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Purification, Kinetic Characterization, and Mapping of the Minimal Catalytic Domain and the Key Polar Groups of *Helicobacter pylori* α-(1,3/1,4)-Fucosyltransferases*[S]

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Bing Ma[‡], Gerald F. Audette[‡], Shuangjun Lin[§], Monica M. Palcic[§], Bart Hazes[‡1], and Diane E. Taylor[‡2]

From the [‡]Department of Medical Microbiology and Immunology, University of Alberta, Edmonton, Alberta T6G 2H7 and the [§]Department of Chemistry, University of Alberta, Edmonton, Alberta T6G 2G2, Canada

The minimal catalytic domain of α-(1,3/1,4)-fucosyltransferases (FucTs) from *Helicobacter pylori* strains NCTC11639 and UA948 was mapped by N- and C-terminal truncations. Only the C terminus could be truncated without significant loss of activity. 11639FucT and UA948FucT contain 10 and 8 heptad repeats, respectively, which connect the catalytic domain with the C-terminal putative amphipathic α-helices. Deletion of all heptad repeats almost completely abolished enzyme activity. Nevertheless, with only one heptad repeat 11639FucT is fully active, whereas UA948FucT is partially active. Removal of the two putative amphipathic α-helices dramatically increased protein expression and solubility, enabling purification with yields of milligrams/liter. Steady-state kinetic analysis of the purified FucTs showed that 11639FucTs possessed slightly tighter binding affinity for both Type II acceptor and GDP-fucose donor than UA948FucT, and its k_{cat} of 2.3 s^{-1} was double that of UA948FucT, which had a k_{cat} value of 1.1 s^{-1} for both Type II and Type I acceptors. UA948FucT strongly favors Type II over the Type I acceptor with a 20-fold difference in acceptor K_m. Sixteen modified Type I and Type II series acceptors were employed to map the molecular determinants of acceptors required for recognition by *H. pylori* α-(1,3/1,4)-FucTs. Deoxygenation at 6-C of the galactose in Type II acceptor caused a 5000-fold decrease in α1,3 activity, whereas in Type I acceptor this completely abolished α1,4 activity, indicating that this hydroxyl group is a key polar group.

Fucosyltransferases (FucTs)[3] catalyze the transfer of the L-fucose moiety from guanosine diphosphate β-L-fucose (GDP-Fuc) to glycoconjugate acceptors in an α1,2-, α1,3-, α1,4-, or α1,6-linkage. Extensive characterizations have been carried out on mammalian α-(1,3/1,4)-FucTs with respect to the domain and amino acids responsible for donor binding (1–7), acceptor specificity (6–14), the key polar groups of acceptors that are essential for enzyme recognition (8, 15–17), and their catalytic mechanism (18–20). In contrast, our knowledge of prokaryotic α-(1,3/1,4)-FucTs is much less advanced (21–25).

Both α-(1,2)- and α-(1,3/1,4)-FucTs have been identified and characterized in *Helicobacter pylori* (21–23, 25–28). These enzymes add fucose to the O antigen of *H. pylori* lipopolysaccharide to produce Lewis antigen structures, a process that is regulated by phase variation at rate of ~0.2–0.5% (29–31). Increasing evidence has shown that Lewis antigens only play a minor role in *H. pylori* colonization and adhesion to the gastric epithelial cells (32–35). Nevertheless, expression of Lewis antigens on *H. pylori* lipopolysaccharide is often associated with severe gastric pathology (36–38). It has also been reported that Lewis antigens bind to dendritic cell-specific ICAM-3-grabbing nonintegrin (DC-SIGN) (39, 40) on dendritic cells modifying the host immune response (40).

Sequence alignments of mammalian and *H. pylori* α-(1,3/1,4)-FucTs show that they share very weak sequence homology (23, 41) that is limited to two small regions designated as α-(1,3)-FucT motifs (22). These motifs have been suggested to be most likely involved in the binding of donor or metal ions (*i.e.* manganese) (22, 42). Mammalian α-(1,3/1,4)-FucTs are typical Golgi-resident type II membrane proteins with a short cytoplasmic N-terminal tail, transmembrane segment, and hypervariable stem domain, preceding the C-terminal catalytic domain. In human FucT III and V, deletion of the N-terminal 61 and 75 amino acids did not lead to significant loss of activity, whereas removal of one or more amino acids at the C terminus in FucT V completely abolished enzyme activity (43). *H. pylori* α-(1,3/1,4)-FucT sequences lack transmembrane domain (21–23). Instead, downstream of the catalytic domain, they contain a short hypervariable region followed by 2–10 heptad repeats (21–23, 41) and a short segment, which is rich in hydrophobic and positive residues that meet the requirements to form two amphipathic α-helices (24). These helices are proposed to function as a membrane anchor with the hydrophobic face embedded in the membrane and the positive charges interacting with phospholipid head groups (24). In most identified *H. pylori* α-(1,3/1,4)-FucTs, the heptad repeat consists of the amino acids DDLRVNY (23, 41). 11639FucT contains 10 heptad repeats with the conserved sequence DDLR(V/I)NY. UA948FucT has 5 internal repeats with this consensus sequence, but the first and the last two heptad repeats consist of the amino acid sequence DDLRRDH (23, 41). It was suggested that the heptad repeat region contains a leucine zipper-like motif responsible for dimerization, which might be essential for enzyme function (21–23). As a result, the C-terminal amphipathic α-helices and the preceding heptad repeat region in *H. pylori* α-(1,3/1,4)-FucTs may be functionally equivalent to the N-terminal transmembrane domain and the stem region of mammalian counterparts, respectively (24). For *H. pylori* FucT from strain NCTC11639, deletion of the C-terminal 115 amino acids, which contain the entire heptad repeat region and its downstream C-terminal tail,

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[1] Scholar of the Alberta Heritage Foundation for Medical Research.

[2] Medical Scientist of the Alberta Heritage Foundation for Medical Research. To whom correspondence should be addressed: Dept. of Medical Microbiology and Immunology, 1-63 Medical Sciences Bldg., University of Alberta, Edmonton, Alberta T6G 2H7, Canada. Tel.: 780-492-4777; Fax: 780-492-7521; E-mail: diane.taylor@ualberta.ca.

[3] The abbreviations used are: FucT, fucosyltransferase; GDP-Fuc, guanosine diphosphate β-L-fucose; ΔFucTs, truncated FucTs; Type I, Galβ1,3GlcNAc; Type II, Galβ1,4GlcNAc; Type I-R, Galβ1,3GlcNAc-O$(CH_2)_8CO_2CH_3$; Type II-R, Galβ1,4GlcNAc-O$(CH_2)_8CO_2CH_3$; IMAC, immobilized metal affinity chromatography; AIEX, anion exchange chromatography; H-Type II, 2-fucosylated Type II; H-Type I, 2-fucosylated Type I; WT, wild type.

including the putative amphipathic α-helices, completely eliminated enzyme activity (21).

Both mammalian and *H. pylori* α-(1,3/1,4)-FucTs display distinct Type I and Type II substrate preferences. Domain swapping and site-directed mutagenesis studies show that acceptor specificity is determined by hypervariable regions that connect the catalytic domain to the membrane anchor, either preceding or following the catalytic domain for mammalian and *H. pylori* FucTs, respectively (12, 13, 24). In particular, a single aromatic residue has been reported to be critical for Type I acceptor specificity for both mammalian and *H. pylori* FucTs (10, 11, 44).

For human α-(1,3/1,4)-FucTs, a panel of modified Type I and Type II series acceptors was used to determine the relative contribution of each hydroxyl group to acceptor binding. For human FucT III, IV, V (16), and FucT VI (8), every hydroxyl group except the 6-OH of galactose and 3-OH or 4-OH of GlcNAc in Type II and Type I acceptor, respectively, tolerated modification. So the 6-OH of galactose and the reactive hydroxyl groups of Type II and Type I acceptors were identified as key polar groups essential for recognition by human α-(1,3/1,4)-FucTs (8, 15–17). For *H. pylori* α-(1,3/1,4)-FucTs, no systematic key polar group mapping has been carried out, and information is limited to two studies of the tolerance to sialylation. The FucT from strain NCTC11637 is able to use 3′-sialyl-Type II but not 6′-sialyl-Type II as an acceptor (22). For *H. pylori* FucT from strain DMS6709, primarily an α-(1,4)-FucT, 3′-sialyl-Type I is an excellent acceptor (25). This suggests that the hydroxyl at C-3 of galactose is not required for the recognition by *H. pylori* α-(1,3/1,4)-FucTs.

FucTs have significant applied interest for the enzymatic synthesis of complex Lewis antigens or as targets for inhibitors. Development of these applications is in great need of crystal structures, but at present, no crystal structure is available for any member of the FucT family. The first challenge to achieve this goal is to isolate large quantities of soluble and stable FucT. In this study, removal of the C-terminal putative amphipathic α-helices increased both protein expression and solubility yet did not significantly reduce the specific enzyme activity. The truncated forms of *H. pylori* α-(1,3/1,4)-FucTs were successfully purified at yields of milligrams/liter and enabled us to determine the detailed kinetic parameters, to map the key polar groups of acceptors essential for enzyme recognition, and to evaluate the potential of recombinant FucTs in enzymatic synthesis of Lewis X and Lewis A structures. These studies greatly expand our knowledge of *H. pylori* FucT function and further demonstrate the great similarity between the mammalian and *H. pylori* enzymes. Finally, the soluble truncated enzymes may also allow us to determine the crystal structures to understand the molecular basis for FucT enzyme catalysis.

EXPERIMENTAL PROCEDURES

Materials—Primers for constructing the truncated FucTs (ΔFucTs) were synthesized by Invitrogen. *Pwo* DNA polymerase and alkaline phosphatase were purchased from Roche Applied Science. pGEM-T vector was obtained from Promega (Madison, WI). Type II-R (Galβ1,4GlcNAc-O-$(CH_2)_8CO_2CH_3$) (**1**), Type I-R (Galβ1,3GlcNAc-O-$(CH_2)_8CO_2CH_3$) (**13**), and Type II series (**2**-**12**) and Type I series (**14**-**18**) modified compounds were kindly provided by Dr. Ole Hindsgaul. Anti-pentahistidine monoclonal antibody and plasmid mini-preparation and midi-preparation kits were purchased from Qiagen (Mississauga, Ontario, Canada). GDP-Fuc and horseradish peroxidase-conjugated goat anti-mouse IgG were from Sigma. GDP-^{3}[H]Fuc (0.1 mCi ml^{-1}, 17.3 Ci $mmol^{-1}$) was obtained from American Radiolabeled Chemicals Inc. (St. Louis, MO). C_{18} reverse-phase cartridges were purchased from Waters. The BCA protein assay kit and Bio-Rad protein assay kit were obtained from Pierce and Bio-Rad, respectively. Coomassie Blue was purchased from Bio-Rad. Nitrocellulose membrane was obtained from Micron Separation Inc. (Westboro, MA). The enhanced chemiluminescence kit was purchased from Amersham Biosciences. BioMax MR film was obtained from Eastman Kodak Co. HisTrap chelating HP nickel-nitrilotriacetic acid columns and Source 30Q anion exchange columns were obtained from GE Healthcare.

Cloning the Truncated FucTs—Wild type (WT) *futA* gene of 11639FucT or UA948FucT was cloned into pGEM-T vector with a His_6 tag fused at the C-terminal end (24). Plasmid DNA harboring the *futA* gene from strains NCTC11639 and UA948 were used as template DNA for PCRs. Primers for the FucTs from NCTC11639 or UA948 were designed to delete different lengths of sequence at either the 5′ or 3′ end. Deletion of the entire (*n*) or *n*-1 heptad repeats but retaining the downstream C-terminal tail was achieved by primer overextension with two steps of sequential PCR (45). The sequences of the primers are available in the Supplemental Material. The codons for six histidine residues were included in the antisense primers when amplifying each truncated FucT. The PCR products were cloned into A/T cloning vector pGEM-T under the control of the T7 promoter. Cloned truncated *futA* genes were sequenced to ensure that no mutation had occurred during their construction.

Expression of Truncated FucTs—Full-length and each truncated FucT (ΔFucT) were expressed in *E. coli* HMS174DE3 cells as described previously (24).

Separation of Soluble and Membrane Fractions of WT and C-terminal Truncated FucTs—500 ml of cells were harvested, resuspended in HEPES buffer (20 mM, pH 7.0, containing 0.5 mM phenylmethylsulfonyl fluoride), and lysed by three passages through a French press (American Instrument Co., Silver Spring, MD) at 12,000 p.s.i. Cell debris was removed by centrifugation at 8,000 × *g* for 10 min, and the supernatant was subsequently centrifuged at 40,000 × *g* for 2 h to separate the soluble fraction (cytosol and periplasm) and the membrane fraction (pellet).

SDS-PAGE Immunoblot Analysis—Cell crude extracts and soluble or membrane fractions were separated by SDS-PAGE. The total protein concentration was determined by the BCA assay using bovine serum albumin as protein standard. FucT expression was detected by immunoblotting with mouse anti-pentahistidine monoclonal antibody (1:1000). The density of the FucT band was quantified and used to normalize the FucT activity as described previously (24).

Radiochemical Assays of ΔFucTs—Enzyme activities of each truncated FucT in crude cell extracts was assayed with donor GDP-Fuc at 200 μM, GDP-^{3}[H]Fuc at 0.2 μM (~60,000 dpm), and Type II-R or Type I-R acceptors at 1.8 or 7.5 mM, respectively (21, 23, 24). The reverse phase C_{18} cartridges were used to isolate the products (containing the hydrophobic aglycone and the acquired radiolabeled fucose moiety) from the unreacted GDP-^{3}[H]Fuc as described previously (46). One milliunit represents the amount of enzyme that converts 1 nmol of acceptor substrate to product/min. The specific activity (milliunit mg^{-1}) was obtained by dividing the enzyme activity (milliunit) by the amount of total protein. The activity below 0.01 milliunit mg^{-1} was considered undetectable. The specific enzyme activity of each FucT was subsequently standardized by FucT expression level, which was determined by immunoblot.

Purification of 11639^{1-441} and $UA948^{1-428}$—The cell lysates from 1 liter of HMS174DE3 11639^{1-441} and $UA948^{1-428}$culture were prepared as described above except the harvested cells were resuspended in loading buffer (20 mM HEPES, pH 7.5, 50 mM imidazole, 300 mM NaCl, 0.5 mM phenylmethylsulfonyl fluoride). Following French press, the cell

debris was removed by centrifugation at 10,000 × *g* for 20 min. The supernatant was retained as a crude cell extract and was subsequently passed through a 0.45-μm filter to remove any remaining particles. The filtered cell extract was loaded onto a HisTrap chelating HP column that had been equilibrated with load buffer (20 mM HEPES, pH 8.0, 300 mM NaCl) on an AKTA purifier (GE Healthcare) at 4 °C. The column was washed with column buffer (20 mM HEPES, pH 8.0, 50 mM NaCl), and the FucT protein was eluted with a 20-column volume of a linear imidazole (0.05–1 M) gradient at a flow rate of 1 ml min^{-1}. Fractions with FucT activity were pooled and loaded onto a Source 30Q anion exchange column, which had been equilibrated with 20 mM HEPES, pH 8.0. The anion exchange column was washed with 20 mM HEPES, pH 8.0, and eluted with a 20-column volume of a linear gradient of NaCl (0–1 M) at a flow rate of 1 ml min^{-1}. As the presence of imidazole in the eluted fractions interferes with the BCA assay, the protein concentration was determined using a Bio-Rad protein assay kit with bovine serum albumin as protein standard. The identity of the purified FucTs was assessed by SDS-PAGE stained with Coomassie Blue, and the purity was quantified by UN-SCAN-IT gel software (Silk Scientific Corp., Orem, UT).

Storage Conditions of Purified FucTs—To determine the best conditions to store purified enzyme (11639FucT$^{1-441}$ and UA948FucT$^{1-428}$), the purified enzyme preparations were stored at 4, −20, and −80 °C, with or without the addition of 20 or 50% glycerol. In addition, a 100-μl aliquot of purified enzyme was lyophilized and kept at −80 °C. At 2-week and 1-, 2-, and 3-month time points, the specific enzyme activities using Type II-R (1.8 mM) from the 10 different storage conditions were determined as described above. Taking the α1,3 activity at day 1 as 100%, the relative levels (%) of enzyme specific activity from the 10 different conditions at the various time points were obtained.

Single Substrate Kinetics of WT and 11639$^{1-441}$ and UA948$^{1-428}$ in Cell Crude Extracts—The acceptor and donor kinetics for the full-length and 11639FucT$^{1-441}$ and UA948FucT$^{1-428}$ in cell crude extracts were carried out as described previously (24, 44). Kinetic parameters were obtained by fitting the initial rate data to the Michaelis-Menten equation using nonlinear regression analysis with Prism 4.0 software (GraphPad, San Diego).

Dual Substrate Kinetics with Purified 11639FucT$^{1-441}$ and UA948$^{1-428}$—Steady-state dual substrate kinetics was carried out on purified 11639FucT$^{1-441}$ and UA948FucT$^{1-428}$ enzyme preparations. Seven different concentrations of the donor and acceptor were used, and GDP-^{3}[H]Fuc (0.8 μM) was included in each reaction. The amount of substrate consumed was less than 15% to ensure linear initial reaction rates. Data were analyzed as described previously (47) by fitting to the rate Equation 1,

$$v = \frac{V_{max}[A][B]}{(K_{ia}K_b + K_a[B] + K_b[A] + [A][B])} \qquad \text{(Eq. 1)}$$

where [*A*] and [*B*] represent the concentration of acceptor and donor, respectively. K_a is the Michaelis constant for acceptor; K_b is the Michaelis constant for donor, and K_{ia} is the dissociation constant for acceptor.

Acceptor Hydroxyl Group Mapping with 11639$^{1-441}$ and UA948$^{1-428}$—Purified 11639FucT$^{1-441}$ and UA948$^{1-428}$ enzyme preparations were used to map the key polar groups of acceptors essential for enzyme recognition. The enzyme activities of using Type II-R (**1**), Type I-R (**13**), Type II series **2**-**12**, and Type I series **14**-**18** modified acceptors were determined with donor GDP-Fuc at 200 μM, GDP-^{3}[H]Fuc at 0.2 μM (~60,000 dpm), and each acceptor at 2 mM. Taking the transfer rates with Type II-R (**1**) or Type I-R (**13**) as 100%, the relative enzyme activity using the Type II series- or Type I series-modified acceptors were obtained and expressed as a percentage, respectively.

Synthesis of Lewis X or Lewis A Using Purified 11639$^{1-441}$ and UA948$^{1-428}$—Type II-R (2 mg, 3.6 μmol) and GDP-Fuc (3.5 mg, 5.4 μmol) were used for Lewis X synthesis with purified 11639FucT$^{1-441}$ in 1.5 ml of HEPES buffer (20 mM, pH 7.0) containing 100 mM NaCl, 35 mM $MgCl_2$, 5 mg ml^{-1} bovine serum albumin, 20 mM $MnCl_2$. The reaction was started by the addition of 180 milliunits of purified 11639FucT$^{1-441}$ and 2 units of alkaline phosphatase. The reaction was gently mixed on an end-over-end shaker at room temperature for 20 h. It has been shown that GDP and GMP are strong inhibitors of human FucT V with K_i values of 0.03 and 0.70 mM, respectively, whereas guanosine has a K_i >10 mM (20). Alkaline phosphatase was therefore added to degrade GDP to GMP and guanosine to minimize the inhibitory effects of the former two compounds. In the same manner, purified UA948FucT$^{1-428}$ was incubated with 5.2 mg (9.4 μmol) of Type II-R and 8.6 mg (13.5 μmol) of GDP-Fuc or 3.2 mg (6 μmol) of Type I-O-R and 5.7 mg (9 μmol) GDP-Fuc for Lewis X or Lewis A synthesis, respectively. The conversion of substrates (Type II and Type I) to products (Lewis X and Lewis A) was monitored by TLC. The synthesized products, Lewis X and Lewis A, were characterized by ^{1}H NMR spectroscopic analysis.

RESULTS

Mapping of the Minimal Catalytic Domain—In an effort to aid FucT overexpression, purification, and structure determination, the minimal catalytic domain of *H. pylori* FucTs was mapped by truncating either the N or the C terminus of 11639FucT and UA948FucT. A schematic diagram depicting the full-length (478 amino acids for 11639FucT and 462 amino acids for UA948FucT) and the ΔFucTs is shown in Fig. 1*A*. 11639FucT is primarily an α-(1,3)-FucT with very low level of α1,4 activity (44), thus only the α1,3 activity of 11639FucT and its truncated constructs was determined. In contrast, both the α1,3 and α1,4 activities of UA948FucT and its truncated mutants were determined by radiochemical assays. The enzyme activity of WT and each ΔFucT was standardized by FucT protein expression level, which was detected by immunoblotting.

To establish if the two putative amphipathic α-helices at the C terminus were essential for enzyme activity, we made deletion constructs lacking these helices and either 5 residues (11639FucT$^{1-447}$, UA948FucT$^{1-434}$) or 11 residues (11639FucT$^{1-441}$, UA948$^{1-428}$) of the 19-residue linker (Fig. 1*A, small white bar*) that connects the helices to the heptad repeats (Fig. 1*A*). The enzyme activities of these four ΔFucTs were about 3–8 times higher than those of the full-length proteins; however, because the protein expression levels were about 10–37 times higher (Fig. 2, *A* and *B*), their specific enzyme activity turned out to be lower than that of the full-length proteins (Fig. 1, *B* and *C*). Protein expression of these four ΔFucTs was increased dramatically in both soluble and membrane fractions as shown in Fig. 2, *C* and *D*. To quantify the distribution of FucT enzyme in the soluble and membrane fractions, we determined the total enzyme activity in each fraction. With full-length FucTs, only 28% (11639FucT) and 20% (UA948FucT) of total enzyme activities were localized in the soluble fraction. For 11639$^{1-441}$ and UA948$^{1-428}$, this increased to 45 and 47%, respectively, indicating that truncation of the C-terminal putative α-helices increased FucT solubility. Nevertheless, a significant amount of active protein was still observed in the membrane fractions when the amphipathic helices were deleted (11639FucT$^{1-447}$, 11639FucT$^{1-441}$, UA948$^{1-434}$, and UA948FucT$^{1-428}$) (Fig. 2, *C* and *D*), suggesting that the C-terminal amphipathic α-helices may not be the sole determinants for membrane association.

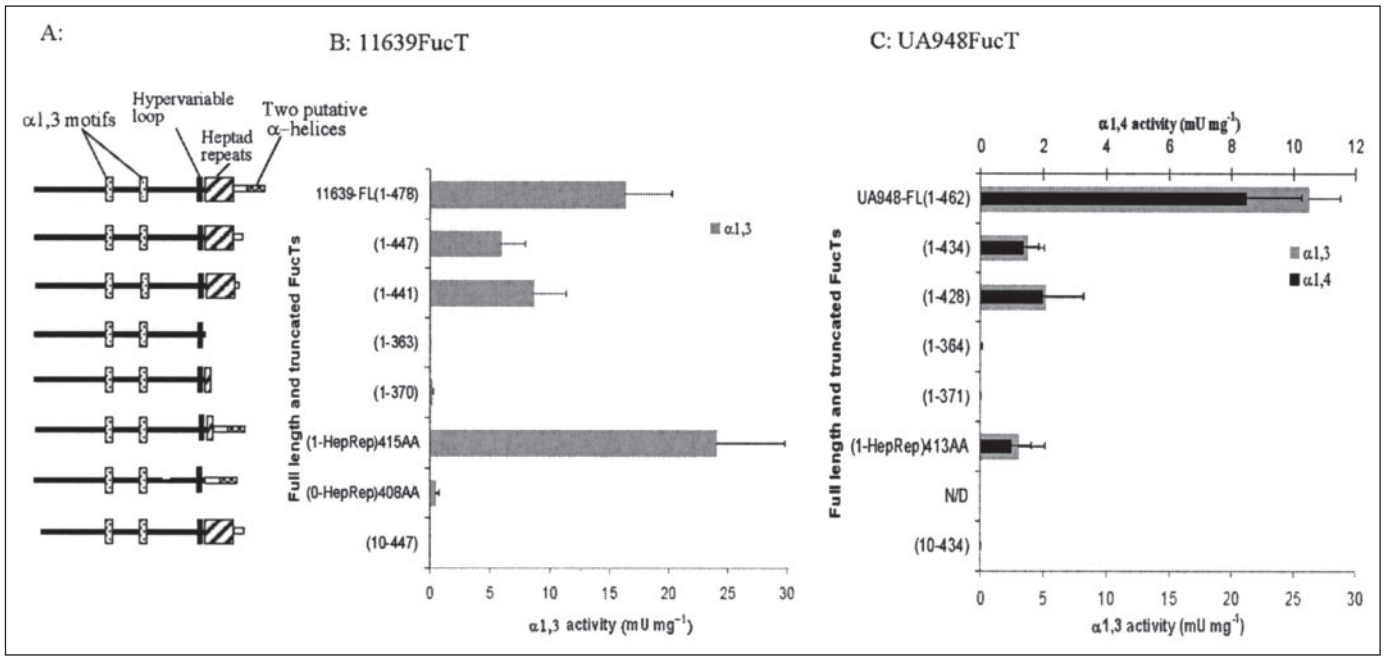

FIGURE 1. **Schematic representation of the truncated FucTs derived from 11639FucT and UA948FucT and their α1,3 and α1,4 activities.** *A,* schematic representation of the full-length and ΔFucTs. The *black bar* represents the domain of FucT preceding the heptad repeat region. The heptad repeat region is shown as a *hatched vertical block.* The two putative C-terminal amphipathic helices are shown as a *checked bar.* The region connecting the heptad repeats and the amphipathic helices is shown as a *small white bar*. Two α-(1,3)-FucT motifs are shown as *narrow dotted vertical blocks*. The hypervariable loop that confers Type I acceptor specificity is shown in a *narrow black vertical block. B* and *C,* the specific enzymatic activities of the full-length (*FL*) and truncated FucTs derived from 11639FucT (*B*) and UA948FucT (*C*), respectively, were standardized by FucT expression levels, which were detected by immunoblotting as shown in Fig. 2, *A* and *B*. The α1,3 activity is shown as *gray bars,* and the α1,4 activity is shown as *black bars.* Each value represents the average of three independent determinations with the standard deviation indicated. 11639FucT is primarily an α-(1,3)-FucT with very low amount of α1,4 activity (44); thus α1,4 activity was not determined. *mU* indicates milliunits.

To determine whether the entire heptad repeat region was required for FucT activity, 11639^{1-363}, $UA948^{1-364}$, and $11639^{0\text{-HepRep}}$ were constructed. After removal of the entire heptad repeat region, these three ΔFucTs exhibited extremely low levels of enzyme activity, although the protein expression was either moderately higher than (11639^{1-363}, $UA948^{1-364}$) or similar to ($11639^{0\text{-HepRep}}$) those of full-length FucTs (Fig. 2). This indicated that the heptad repeat region is essential for enzyme activity.

To determine whether a shorter heptad repeat region was sufficient for conferring enzyme activity, constructs 11639^{1-370} and $UA948^{1-371}$ were made, which are truncated immediately after the first heptad repeat. In addition, $11639^{1\text{-HepRep}}$ and $UA948^{1\text{-HepRep}}$ that contain one heptad repeat plus the downstream 19 amino acids linker and the two putative amphipathic α-helices were also made (Fig. 1*A*). Constructs 11639^{1-370} and $UA948^{1-371}$ had little enzyme activity (Fig. 1, *B* and *C*). Remarkably, $11639^{1\text{-HepRep}}$ displayed enzyme activity comparable with that of the full-length protein. This suggests that a much shorter "stem" region of just one heptad repeat is sufficient for full activity of 11639FucT (Fig. 1*B*). In contrast, UA948FucT with one heptad repeat ($UA948^{1\text{-HepRep}}$) exhibited partial activity compared with that of the full-length enzyme (Fig. 1*C*). We consistently observed that $UA948^{1\text{-HepRep}}$ displayed a lower protein expression level than the full-length protein and the other constructs (Fig. 2*B*), suggesting that this construct was either poorly expressed or the protein was unstable. $UA948^{0\text{-HepRep}}$ was also made; however, it not possible to characterize this mutant because of the lack of protein expression. It is possible that deletion of the entire heptad repeat region of UA948FucT causes folding or degradation problems that prevent proper expression.

Finally, to determine whether or not the N terminus of *H. pylori* FucTs is essential for function, 11639^{10-447} and $UA948^{10-434}$ were constructed by deleting 10 amino acids at the N terminus of 11639^{1-447} and $UA948^{1-434}$, respectively. These two truncated constructs completely lost enzyme activity (Fig. 1, *B* and *C*), despite the fact that both were expressed at a similar level as full-length enzymes (Fig. 2, *A* and *B*). This demonstrates the necessity of the N-terminal residues for enzyme function.

Purification of 11639^{1-441} and $UA948^{1-428}$—Removal of the C-terminal helices greatly improved protein expression and solubility while maintaining enzyme activity at a significant level (Figs. 1 and 2). Therefore, $11639FucT^{1-441}$ and $UA948FucT^{1-428}$ with a His_6 tag at the C terminus were purified by a sequence of immobilized metal affinity chromatography (IMAC) and anion exchange chromatography (AIEX). The calculated molecular masses of $11639FucT^{1-441}$ and $UA948FucT^{1-428}$ are 52.5 and 51.2 kDa, respectively. The FucT bands were visible at the predicted position but did not stand out on a Coomassie Blue-stained SDS-polyacrylamide gel prior to purification (Fig. 3, *lanes 1* and *4*). The two-step purification resulted in 78.2 and 79.7% purity for 11639^{1-441} and $UA948^{1-428}$, respectively (Fig. 3). A minor doublet band is sometimes observed in constructs 11639^{1-447} and 11639^{1-441} either before (Fig. 2*C*) or after purification (Fig. 3, *lane 2*). The nature of this band is unknown, but because it runs at higher molecular weight than the main band, it is likely because of a post-translational change (*e.g.* oxidation of cysteine). As there was only small amount of this material, any effects on our measurements should be negligible.

From 1-liter cultures, 6.7 and 4.4 mg of FucT protein was isolated, and the enzyme-specific activity was increased from 0.05 to 3.7 units mg^{-1} (77-fold) and 2.8 units mg^{-1} (52-fold) for $11639FucT^{1-441}$ and $UA948FucT^{1-428}$, respectively. It is worth noting that $UA948^{1-428}$ was rather unstable after the IMAC column and would precipitate if the fractions were left at 4–8 °C for 30–60 min. Therefore, the FucT fractions eluted from the IMAC column were loaded immediately onto the

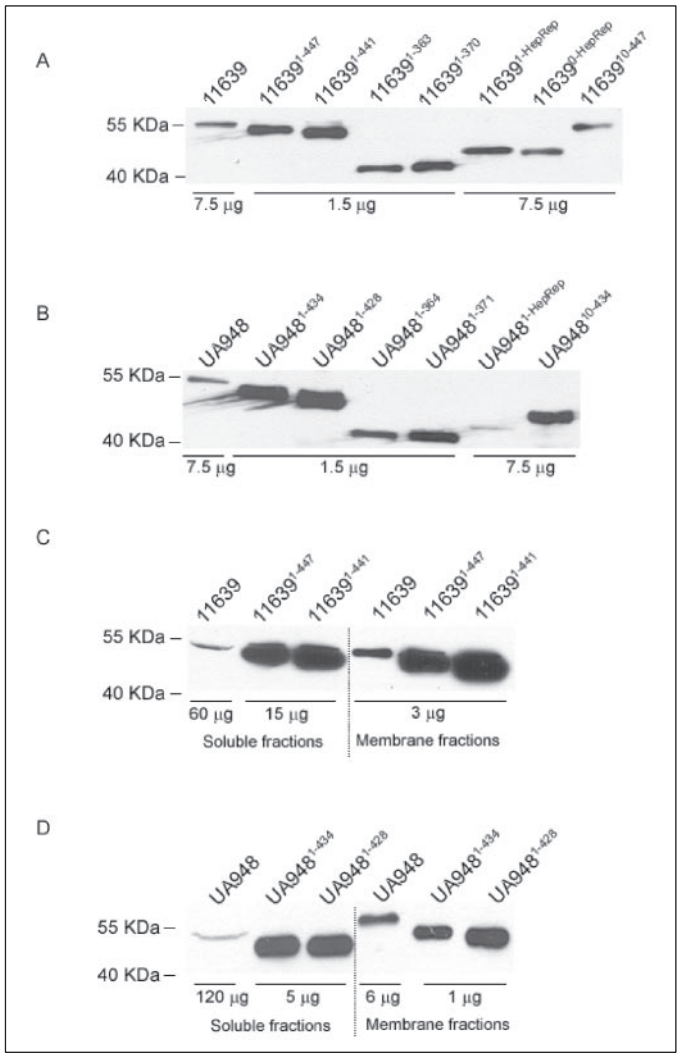

FIGURE 2. **Immunoblot of full-length and truncated FucTs.** The full-length and truncated FucTs with His_6 tag were expressed in *E. coli* HMS174DE3 cells with induction using 1 mM isopropyl β-D-thiogalactopyranoside and growth at 30 °C for 4–5 h. The FucT protein was detected with mouse anti-pentahistidine monoclonal antibody. *A–D,* the amount of total protein loaded is shown at the bottom of each lane. *C* and *D,* soluble and membrane fractions are also labeled.

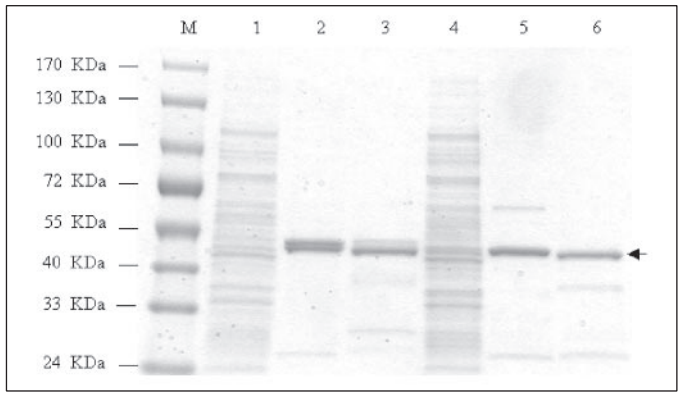

FIGURE 3. **SDS-PAGE of FucT proteins before and after purification.** The gel was stained with Coomassie Blue. Total protein of 6 μg (crude extracts, *lanes 1* and *4*) or 3 μg (purified fractions, *lanes 2, 3, 5,* and *6*) was loaded. *Lanes 1–3*, 11639FucT$^{1-441}$; *lanes 4–6*, UA948FucT$^{1-428}$. *Lanes 1* and *4*, cell crude extracts *lanes 2* and *5*, pooled IMAC purification fractions; *lanes 3* and *6*, pooled AIEX purification fractions. The *arrow* indicates the positions of 11639$^{1-441}$ and UA948$^{1-428}$ on the blot. *M*, protein molecular weight marker.

AIEX column. Even so, a significant drop in both total and specific enzyme activity was observed during the AIEX purification procedure.

Storage and Stability—To determine the best storage method for the purified FucTs, the α1,3 activity was monitored by radiochemical assays following enzyme storage after 2 weeks and 1, 2, or 3 months at three different temperatures (4, −20, and −80 °C) with different concentrations of glycerol (0, 20, or 50%). In addition, one sample was lyophilized and stored at −80 °C. Lyophilized 11639$^{1-441}$ and UA948$^{1-428}$ lost 38 and 23% activity after 3 months, respectively. Addition of glycerol (20 or 50%) significantly stabilized the enzyme preparations at both 4 and −20 °C, with little loss of activity after 3 months. FucT preparations were most stable at −80 °C with or without glycerol supplementation; thus this condition has been adopted as our storage protocol.

Single Substrate Kinetics of WT and 11639$^{1-441}$ and UA948$^{1-428}$ in Crude Extracts—Removal of the C-terminal putative amphipathic helices facilitated the overexpression and purification of *H. pylori* α-(1,3/1,4)-FucTs; however, it was not known whether the truncation affected the kinetic parameters for the enzyme. The single substrate kinetics of full-length and truncated constructs 11639$^{1-441}$ and UA948$^{1-428}$ in cell crude extracts were characterized and compared.

The acceptor kinetic data showed that truncation of the C terminus did not cause any modification of the Type II acceptor kinetics for 11639FucT; however, although the acceptor K_m remained unchanged for UA948FucT, the V_{max} decreased by 50% (Table 1). With Type I acceptor, UA948$^{1-428}$ possessed a 2-fold tighter acceptor binding affinity, which exactly compensated for the 2-fold reduction of the V_{max} value when calculating the catalytic efficiency (V_{max}/K_m) of the enzyme. Notably, the C-terminal truncation improved donor binding by about 3-fold with both Type II and Type I acceptors for both FucTs.

Dual Substrate Kinetics of Purified 11639$^{1-441}$ and UA948$^{1-428}$—The success in purifying milligrams/liter of 11639$^{1-441}$ and UA948$^{1-428}$ allowed us to perform dual substrate kinetics with seven different concentrations of the donor GDP-Fuc and seven different concentrations of acceptor (Type II or Type I). GDP-^{3}[H]Fuc at 0.8 μM was included in each reaction. The kinetic parameters are shown in Table 2. The acceptor and donor K_m values for fucose transfer to Type II acceptor by 11639FucT$^{1-441}$ and UA948FucT$^{1-428}$ are similar, with those of 11639FucT$^{1-441}$ just slightly lower (Table 2). The K_m values of 11639FucT$^{1-441}$ and UA948FucT$^{1-428}$ are 2.3 and 1.1 s^{-1}, respectively, which give rise to the catalytic efficiency parameters (k_{cat}/K_a and k_{cat}/K_b) of 11639FucT$^{1-441}$, with Type II acceptor being ~3-fold those of UA948FucT$^{1-428}$. UA948FucT possessed the same k_{cat} values (1.1 s^{-1}) for Type II and Type I acceptors. However, the much lower K_m value for the Type II acceptor resulted in a 28-fold higher k_{cat}/K_a with Type II compared with that of the Type I acceptor; therefore, UA948FucT strongly favors Type II over Type I acceptor. For the donor GDP-Fuc, UA948FucT$^{1-428}$ displayed a slightly tighter binding when fucose was transferred to Type I *versus* Type II acceptor. The acceptor and donor K_m values obtained from dual substrate kinetics with the purified enzyme (Table 2) are lower than those obtained from single substrate kinetics with cell crude extracts (Table 1), except for the K_m using Type I acceptor for UA948$^{1-428}$.

To determine the kinetic mechanisms of 11639$^{1-441}$ and UA948$^{1-428}$, a pattern of double-reciprocal plots for velocity and concentration of either substrate at a series of fixed concentrations of the second substrate was produced. The resulting family of intersecting lines (data not shown) excludes a double displacement (ping-pong) mechanism. Therefore, we can conclude that the catalytic mechanism of *H. pylori* FucTs is sequential and that both substrates bind to the enzyme before any product is released.

TABLE 1

The acceptor and donor kinetic parameters of wild type and truncated FucTs

Acceptor kinetics were determined using 0.03–2 mM Type II-R or 0.4–25 mM Type I-R with GDP-Fuc at 200 μM and GDP-[^{3}H]Fuc at 0.2 μM. Donor kinetics were determined using 3–200 μM GDP-Fuc with Type II-R at 2 mM or Type I-R at 15 mM and GDP-[^{3}H]Fuc at 0.8 μM.

Full-length and truncated[a]	Type II-R[b]			Type I-R[c]			K_m for GDP-Fuc	
	K_m	V_{max}[d]	V_{max}/K_m[d]	K_m	V_{max}	V_{max}/K_m[d]	Type II-R[b]	Type I-R[e]
	mM			mM			μM	
11639FucT	0.37 ± 0.03	11.8 ± 0.3	31.9	–[f]	–[f]	–[f]	44.7 ± 5.5	–[f]
UA948FucT	1.5 ± 0.2	29.0 ± 1.8	19.3	13.4 ± 1.4	11.7 ± 0.6	0.87	56.0 ± 10.1	33.7 ± 7.8
11639^{1-441}	0.39 ± 0.07	12.0 ± 0.8	30.8	–[f]	–[f]	–[f]	15.6 ± 2.4	–[f]
$UA948^{1-428}$	1.7 ± 0.2	16.1 ± 1.0	9.5	5.7 ± 0.5	5.0 ± 0.3	0.88	17.6 ± 3.7	11.9 ± 0.8

[a] All FucTs possessed a His_6 tag at the C terminus.
[b] Type II-R, Galβ1,4GlcNAc-O$(CH_2)_8CO_2CH_3$.
[c] V_{max} (milliunits mg^{-1}) was standardized based on FucT expression quantified by immunoblot.
[d] V_{max}/K_m, milliunits mg^{-1} mM^{-1}.
[e] Type I-R, Galβ1,3GlcNAc-O$(CH_2)_8CO_2CH_3$.
[f] – indicates not determined because of the very low level of α1,4 activity; thus the kinetic parameters could not be obtained with confidence.

TABLE 2

Dual substrate kinetic parameters of the purified truncated $11639FucT^{1-441}$ and $UA948FucT^{1-428}$ with donor (GDP-Fuc) and acceptor (Type II or Type I)

k_{cat} is the catalytic turnover rate; K_a is the Michaelis constant for acceptor; K_b is the Michaelis constant for donor; K_{ia} is the dissociation constant for acceptor; K_{ib} is the dissociation constant for donor. The α values reflect the effect of binding one substrate to the binding of the other.

	$11639FucT^{1-441}$ [a,b]	$UA948FucT^{1-428}$ [a]	
	Type II	Type II	Type I
k_{cat} (s^{-1})	2.3 ± 0.1	1.1 ± 0.1	1.1 ± 0.1
K_a (μM/mM)	305 ± 32 μM	397 ± 79 μM	8.3 ± 0.7 mM
K_b (μM)	10.4 ± 1.5	14.4 ± 3.7	8.1 ± 1.7
K_{ia} (μM/mM)	95 ± 16 μM	127 ± 6 μM	2.2 ± 0.4 mM
K_{ib} (μM)	3.2 ± 1.2	5.3 ± 0.9	1.6 ± 0.4
α	3.3 ± 0.1	2.9 ± 0.2	4.4 ± 0.6
k_{cat}/K_a ($\times 10^3$ s^{-1} M^{-1})	7.5	2.8	0.1
k_{cat}/K_b ($\times 10^3$ s^{-1} M^{-1})	221	76	136
$k_{cat}/K_{ia}K_b$ ($\times 10^9$ s^{-1} M^{-1})	2.3	0.6	0.06

[a] Both FucT enzymes have a His_6 tag at the C terminus.
[b] 11639FucT is primarily an α-(1,3)-FucT with trace amount of α-1,4 activity (44); thus the kinetic parameters of 11639^{1-441} with Type I acceptor were not determined.

Acceptor Hydroxyl Group Mapping with H. pylori FucTs—To map the molecular determinants in Type II and Type I acceptors that are essential for recognition by *H. pylori* α-(1,3/1,4)-FucTs, 16 synthetic acceptors with modifications at the hydroxyl and *N*-acetamido groups were employed. Because of the limited availability of the compounds, the enzyme activities were determined using 2 mM of each acceptor. In comparison to the transfer rate with unmodified Type II acceptor (**1**) or Type I acceptor (**13**), the relative rates (%) of transfer for each modified acceptor were obtained (Table 3).

Purified 11639^{1-441} and $UA948^{1-428}$ FucTs exhibited variable levels of enzyme activity using 12 different Type II series acceptors (Table 3). Substitution of the hydroxyl group at C-3 of the galactose with a sulfate group (**2**) and replacement of the hydroxyl group at C-2 of the galactose with fucose (**3**) resulted in enhanced or comparable rates of transfer for 11639^{1-441} and $UA948^{1-428}$, respectively, relative to the unmodified acceptor (**1**). Addition of a bulky sialic acid moiety to the C-3 of galactose (**4**) greatly reduced the rate of transfer, particularly for UA948FucT (Table 3). Modifications at the C-4 position with deoxygenation (**5**) reduced the activity about 4- and 13-fold for 11639FucT and UA948FucT, respectively (Table 3). The most dramatic impact was observed by modifications at C-6. When this hydroxyl was deoxygenated, compound (**7**) became a very poor substrate for both 11639^{1-441} and $UA948^{1-428}$ with 0.02% of transfer rate relative to the unmodified Type II acceptor (**1**). Replacement of the C-6 hydroxyl group by a sulfate group (**8**) gave a slightly improved transfer rate at 2 and 0.04%, compared with compound (**7**) for 11639^{1-441} and $UA948^{1-428}$, respectively. Such dramatic decreases in activity qualify the hydroxyl at C-6 of galactose in Type II acceptor as a key polar group required for enzyme recognition (Fig. 4). In general, 11639^{1-441} tolerated modifications on the terminal galactose moiety of Type II acceptor (compounds **2** and **4–6**) better than $UA948^{1-428}$. This may be due to the lower K_m for Type II acceptor of 11639FucT than that of UA984FucT (Table 1 and Table 2).

Modifications of the GlcNAc moiety in Type II acceptor greatly reduced the transfer rate for both enzymes. Phosphorylation of the hydroxyl group at C-6 of the GlcNAc (**8**) caused a large decrease in activity for both 11639^{1-441} and $UA948^{1-428}$. The lack of an *N*-acetamido group (**9**) also severely impaired activity. Modification of compound (**9**) by adding a galactose at C-3 (**10**) resulted in a better acceptor. Apparently, the protein can sterically accommodate additions as large as a galactose to the C-3 position, and the enhanced enzyme activity likely results from favorable interactions of this moiety with the FucT protein. Modification of compound (**9**) by adding a galactose at C-4 (**11**) did not show the same increase in activity as compound (**10**), but neither did it reduce activity completely. Again, addition at the C-4 position appears to be sterically tolerated, suggesting that the C-4 hydroxyl points away from the FucT protein.

We also deoxygenated the C-3 of the GlcNAc of Type II substrate to evaluate if compound (**12**) could function as an inhibitor. When 4.4 mM of compound (**12**) and 55.6 μM of substrate (**1**) were included in the reaction, 42.5 and 28.3% inhibition was observed for 11639^{1-441} and $UA948^{1-428}$, respectively. The limited availability of compound (**12**) precluded us from performing a complete kinetic study to obtain an accurate K_i. Nevertheless, assuming that the inhibition is competitive, a K_i of 5.0 and 9.8 mM could be calculated for 11639^{1-441} and $UA948^{1-428}$, respectively, using the relationship of $i = 100\ [I]/([I] + K_i\{1 + [S]/K_m\})$, where i is the % inhibition; [I] is the inhibitor concentration; [*S*] is the

TABLE 3

Acceptor specificity of the purified recombinant *H. pylori* FucTs for 18 Type II and Type I series oligosaccharides

The relative rates of fucose transfer with Type II series compounds are expressed as a percentage of incorporation with compound (**1**) for 11639^{1-441} and $UA948^{1-428}$ FucTs, respectively. The relative rates with Type I series compounds are expressed as a percentage of incorporation with compound (**13**) for purified $UA948^{1-428}$ FucT. Each acceptor was used at 2 mM. The specific enzyme activities with compound (**1**) for 11639^{1-441} and UA948FucT$^{1-428}$ are 2.9 and 8.6 units mg^{-1}, respectively, and with compound (**13**) for $UA948^{1-428}$ is 1.5 units mg^{-1}. These rates correspond to the relative activity of 100%.

Substrate[a]	Relative activity	
	11639^{1-441} [b]	$UA948^{1-428}$ [b]
	%	
Type II series		
(1) Galβ1,4GlcNAc	100	100
(2) 3-Sulfo-Galβ1,4GlcNAc	135	72
(3) Fucα1,2Galβ1,4GlcNAc	115	99
(4) NeuAcα2,3Galβ1,4GlcNAc	32	1
(5) 4-Deoxy-Galβ1,4GlcNAc	28	8
(6) 6-Deoxy-Galβ1,4GlcNAc	0.02	0.02
(7) 6-Sulfo-Galβ1,4GlcNAc	2	0.04
(8) Galβ1,4–6-Phospho-GlcNAc	6	0.4
(9) Galβ1,4Glc	0.4	1
(10) Galα1,3Galβ1,4Glc	3	2
(11) Galα1,4Galβ1,4Glc	0.1	0.04
(12) Galβ1,4–3-Deoxy-GlcNAc	–[c]	–
Type I series		
(13) Galβ1,3GlcNAc	ND[d]	100
(14) 3-Sulfo-Galβ1,3GlcNAc	ND	40
(15) Fucα1,2Galβ1,3GlcNAc	ND	34
(16) NeuAcα2,3Galβ1,3GlcNAc	ND	0.05
(17) 6-Deoxy-Galβ1,3GlcNAc	ND	–
(18) Galβ1,3–4-deoxy-GlcNAc	ND	–

[a] All Type I and Type II series acceptors have an aglycone (β-*O*-$(CH_2)_8CO_2CH_3$) at C-1 of the GlcNAc moiety.
[b] FucT enzymes have a His_6 tag at the C terminus.
[c] – indicates activity lower than 0.01 milliunit mg^{-1} was considered undetectable.
[d] ND indicates not determined.

acceptor concentration, and K_m is the acceptor K_m for compound (**1**). The high K_i value of compound (**12**) argues for an important role for OH-3 of Type II acceptor in recognition by 11639FucT and UA948FucT. Such weak inhibition has also been observed for FucTs isolated from human milk when 4-OH of GlcNAc in Type I acceptor was deoxygenated (15, 17).

Five modified Type I series analogues were also utilized to map the hydroxyl groups in Type I acceptor that are required for recognition by $UA948^{1-428}$ (Table 3). Substitutions of the hydroxyl group at C-3 of the galactose with a sulfate group (**14**) and the C-2 of the galactose with a fucose group (**15**) were relatively well tolerated. In contrast, the replacement of C-3 of the galactose with sialic acid (**16**) created a very poor acceptor. Notably, deoxygenation at C-6 of the galactose (**17**) rendered the compound completely inactive, indicating that the hydroxyl group at C-6 of the galactose in Type I acceptor is absolutely required for recognition by $UA948^{1-428}$. As expected, deoxygenation at C-4 of GlcNAc in Type I structure (**18**) made the compound unable to function as an acceptor. When Type I analogue (**18**) at 4.4 mM was evaluated as an inhibitor with (**13**) being included in the reaction at 55.6 μM as an acceptor, no inhibition was observed. This indicates that the reactive hydroxyl in Type I acceptor is crucial for enzyme recognition.

Synthesis of Lewis X and Lewis A Using Purified 11639^{1-441} and $UA948^{1-428}$—Purified 11639^{1-441} and $UA948^{1-428}$ were used for enzymatic synthesis of Lewis X and Lewis A. The conversion rate from substrate to product in each reaction was monitored by TLC ($CHCl_3$/MeOH/water = 70:35:2). When TLC showed the complete disappearance of the substrate and the formation of a single product, the reaction was terminated. The product was isolated by column chromatography on a reverse-phase C_{18} cartridge and was subsequently lyophilized, with yields ranging from 87 to 94%. The products (Lewis X and Lewis A) and the starting substrates (Type II and Type I) were analyzed by ^1H NMR spectroscopy, and the parameters of the feature peaks are given in the Supplemental Material. The chemical shifts for products are identical to literature values for Lewis X and Lewis A (48, 49).

DISCUSSION

Our current data demonstrate that the C terminus, but not the N terminus, of *H. pylori* α-(1,3/1,4)-FucTs can be truncated without significant loss in activity. This is in contrast to mammalian α-(1,3/1,4)-FucTs where truncation of the C terminus, but not the N terminus, abolishes activity. This supports our inverted domain model, in which *H. pylori* α-(1,3/1,4)-FucTs contain the C-terminal heptad repeat region followed by two putative amphipathic α-helices that are functionally equivalent to the N-terminal stem and transmembrane regions, respectively, in mammalian α-(1,3/1,4)-FucTs (24).

Removal of the putative α-helices at the C terminus did not significantly reduce the specific enzyme activity, indicating that the amphipathic helices are not absolutely required for conferring activity. Remarkably, the truncation greatly increased protein expression and protein solubility (Fig. 2), which enabled us to purify a significant amount of soluble active enzyme for detailed kinetic characterization and key polar group mapping. Previously, similar increases of protein expression have been observed by deleting the C-terminal putative amphipathic helices in α1,4-galactosyltransferase LgtC from *Neisseria meningitidis* (50) and the sialyltransferase CstII from *Campylobacter jejuni* (51). It is worth noting that the increase in protein solubility after removal of the putative amphipathic helices supports their role as a membrane anchor. Nevertheless, a substantial portion of protein still localized to the membrane fraction when they were deleted (Fig. 2, *C* and *D*). This remained the case even when the preceding heptad repeats were also removed (data not shown). It is possible that the catalytic domain possesses an additional membrane-attachment region, which remains to be identified. Alternatively, protein overexpression may have caused membrane association because of the exposed hydrophobicity of some partially unfolded proteins.

In *H. pylori* FucTs, the putative membrane anchor is connected to the catalytic domain by a series of 2–10 leucine zipper-like heptad repeats (41), which has been speculated to mediate formation of a functional dimeric enzyme (21–23). Deletion of the entire heptad repeat region (11639^{1-370}, $UA948^{1-371}$, and $11639^{0\text{-HepRep}}$) almost completely abolished enzyme activity for both 11639FucT and UA948FucT, suggesting that the heptad repeat region is essential for enzyme activity. Because as few as two heptad repeats have been observed in nature (41), it is not surprising to observe the full or partial enzyme activity present in the single heptad repeat constructs, $11639^{1\text{-HepRep}}$ and $UA948^{1\text{-HepRep}}$, respectively. Most interestingly, the amphipathic helices are necessary for function when the repeat region is reduced to one heptad repeat (Fig. 1). In addition, the 11639FucT heptad repeats truncations are typically more stable and have higher activity than the equivalently truncated UA948 FucT constructs (Fig. 1). These observations suggest a more complex model where the C-terminal amphipathic helices, heptad repeats, and perhaps the catalytic domain all contribute to dimerization; thus full activity is obtained as long as the net interaction energy is high enough to form a stable dimer. We are currently pursuing this hypothesis, and our preliminary dynamic light scattering data has confirmed that 11639^{1-441} indeed forms a dimer (results to be published elsewhere). A functional requirement for dimerization has not yet been reported for any other α-(1,3/1,4)-FucTs, but dimeric forms of human FucT VII exist within cells (52).

The dual substrate kinetic analysis shows that *H. pylori* α-(1,3/1,4)-

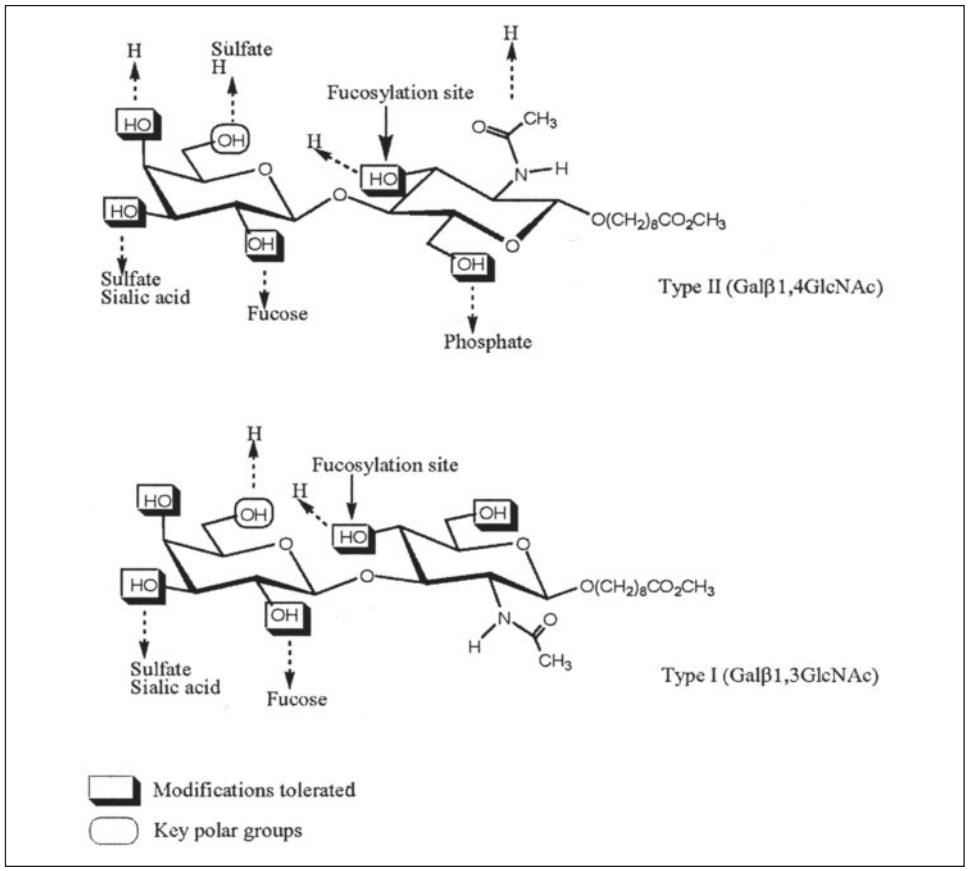

FIGURE 4. **Structure of the Type II and Type I acceptors and the key polar group mapping for *H. pylori* α-(1,3/1,4)-FucTs.** The *broken arrows* point to the modifications in our current study.

FucTs possess comparable kinetic parameters to their mammalian counterparts. Specifically, the donor GDP-Fuc K_m values for *H. pylori* α-(1,3/1,4)-FucTs are similar to those for human FucT III, IV, V (16), and VI (8), and the K_m values for Type II-R acceptor ($R=O(CH2)_8CO_2CH_3$) are similar to that of FucT VI (8). With Type II and Type I acceptors that do not contain the hydrophobic linker ($-O(CH2)_8CO_2CH_3$), human FucT III and V display a low k_{cat} ranging from 5.2 to 9.3 min^{-1} (13). The newly characterized *H. pylori* α-(1,3/1,4)-FucT from strain DMS6709, which is primarily an α-(1,4)-FucT, possesses a similar acceptor K_m (313 μM) for Type II-R acceptor, a lower K_m (114 μM) for Type I acceptor, and a relatively low K_m for donor (5.73 μM). Compared with UA948FucT, DMS6709FucT has 24-fold higher k_{cat} for Type I acceptor (26 s^{-1}) and 16-fold lower K_m for Type II acceptor (0.067 s^{-1}) (25). It is interesting that the Type II acceptor preference in UA948FucT is because of the 21-fold lower K_m for Type II than that for Type I, but their k_{cat} values are identical, whereas DMS6709FucT favoring Type I acceptor results from the 388-fold lower k_{cat} but only <3-fold lower K_m values for Type I compared with Type II acceptor.

Similar to human FucT V (20), the pattern of the double-reciprocal plots for velocity with concentration of either substrate at a series of fixed concentrations of the second suggests that *H. pylori* α-(1,3/1,4)-FucTs catalyze fucose transfer following a sequential mechanism. Our current study cannot distinguish whether the substrate binding is random or ordered, and if it is ordered, whether the acceptor or the donor binds first. In the process of optimizing the purification protocol, we observed that both 11639^{1-441} and $UA948^{1-428}$ bind to GDP-hexanolamine-Sepharose affinity chromatography support columns but not to immobilized acceptor-based affinity columns,[4] indicating that *H. pylori* FucTs most likely binds to the donor first, as has been observed for most other glycosyltransferases (53). Further detailed kinetic and structural studies are required to fully resolve this issue.

It has been shown that mammalian α-(1,3/1,4)-FucTs are able to use 3′-sialylated Type II or 3′-sialylated Type I acceptors to synthesize 3′-sialylated-Lewis X or 3′-sialylated-Lewis A, respectively, but with variable efficiencies. Our hydroxyl group mapping data demonstrates that UA948FucT tolerates sialylation very poorly. Compared with mammalian α-(1,3/1,4)-FucTs, *H. pylori* 11639FucT and 11637FucT resemble human FucT VI (8) in their preference for neutral Type II acceptor and tolerance for 3′-sialylation, whereas UA948FucT behaves more like human FucT IV and V for its α1,3 activity and α1,4 activity, respectively. Human FucT IV transfers fucose much more efficiently to neutral Type II acceptors than those that are sialylated (6). FucT V prefers both fucosylated and sialylated Type II acceptor; however, it does not favor sialylated Type I structures (16). The α-(1,3/1,4)-FucT from strain DMS6709 is more like human FucT III as both of them prefer the sialylated Type I over the unmodified Type I acceptor (25).

Although it appears most *H. pylori* FucTs can utilize sialylated acceptors, only very few *H. pylori* isolates (2 out 94) were found to express 3′-sialylated-Lewis X (54), such as strain P466 (55). This is most likely because of the absence of a functional α-2,3-sialyltransferase, which has not yet been identified in any *H. pylori* strains. It would be of interest to determine whether *H. pylori* α-2,3-sialyltransferase is able to use the 2′-fucosylated Type II or 2′-fucosylated Type I as acceptors, like the α-2,3-sialyltransferase from myxoma virus (51), or if sialylation has to precede to fucosylation as has been reported for mammalian α-2,3-sialyltransferases (56).

Our hydroxyl group mapping data show that the 2-fucosylated

[4] B. Ma, M. M. Palcic, B. Hazes, and D. E. Taylor, unpublished data.

Type II (H-Type II (**3**)) is an excellent acceptor for both *H. pylori* FucTs, and 2-fucosylated Type I (H-Type I (**15**)) is also a fairly good acceptor for UA948$^{1-428}$. This challenges the notion that the difucosylated Lewis antigens (Lewis Y and Lewis B) in *H. pylori* are synthesized predominantly via subterminal fucosylation (in α1,3- or α1,4-linkage) followed by terminal fucosylation (in α1,2-linkage) (57). Martin *et al.* (22) showed that 11637FucT was unable to use Fuc(α1,2)Galβ1,4*Glc* as an acceptor. Because the lack of the *N*-acetamido group in Type II structure severely impaired enzyme activity (Table 3), one cannot conclude that 11637FucT is unable to use Fuc(α1,2)Galβ1,4*GlcNAc* (H-Type II) as an acceptor. The α1,2-FucTs from *H. pylori* were reported to use both the unfucosylated (Type II and Type I) and the fucosylated (Lewis X and Lewis A) structures as acceptors (28). Therefore, we propose that the difucosylated Lewis antigens in *H. pylori* can be synthesized via either route as follows: α-(1,3/1,4)-fucosylation followed by α1,2-fucosylation or vice versa. The route used predominantly *in vivo* will depend on activity and expression levels as well as the substrate preferences of α-(1,3/1,4)-FucTs and α1,2-FucT in a particular *H. pylori* strain. In *H. pylori* strains NCTC11639 and UA948, this is not an issue because their *futC* gene does not encode a functional α1,2-FucT protein (23, 57).

The key polar group mapping data also show that phosphorylation of the GlcNAc at 6-C position (**8**) caused a dramatic reduction in activity for both enzymes, and the lack of the *N*-acetamido group (**9**) also generated a dramatic decrease in activity, by 250-fold for 11639FucT and by 100-fold for UA948FucT. This implies that the 6-OH and the acetamido group in GlcNAc of Type II acceptor contribute to optimal enzyme activity. It has been shown previously that the acetamido group in Type II acceptor is required for optimal activity of human FucT III, IV, and V (16).

Our key polar group mapping data suggest that the hydroxyl at C-6 of galactose in Type I and Type II acceptors is a key polar group essential for recognition by *H. pylori* α-(1,3/1,4)-FucTs (Fig. 4). For the reactive hydroxyl group in Type II acceptor, when it is deoxygenated, the compound functions as a weak inhibitor, but the relatively high K_i value suggests that this hydroxyl does play an important role in enzyme binding. When OH-4 of the GlcNAc moiety in Type I acceptor is deoxygenated, we did not observe any inhibition. As UA948$^{1-428}$ possesses a relatively high K_m value for Type I acceptor, the actual K_i value is hard to quantify. However, if we assume competitive inhibition and a 5% detection limit of the assay, we estimate the K_i to be 83 mM or greater. A similar sensitivity pattern has been reported for mammalian α-(1,3/1,4)-FucTs (8, 15–17). This suggests that both mammalian and *H. pylori* α-(1,3/1,4)-FucTs bind the Type II and Type I compounds in a similar manner. In our previous work, we have proposed that the GlcNAc moiety in Type I structure is rotated by 180° relative to its location in Type II compound, bringing the 4-OH of GlcNAc in Type I to the same position as the 3-OH of GlcNAc in Type II (44). The 6-CH_2OH and acetamido groups in Type I are also rotated 180° relative to those in Type II structures. This hypothesis is strengthened by NMR studies of Lewis X and Lewis A trisaccharides, where the fucose and galactose in both compounds occupy very similar relative positions with the major difference being the opposite orientation of their GlcNAc moiety (58). We noticed that the absence of the acetamido group (**9**) is the only modification that causes a less dramatic effect on the transfer rate observed by UA948FucT as compared with 11639FucT (Table 3). This suggests that the *N*-acetamido group contributes less to recognition by UA948FucT than 11639FucT. This may at least partially explain why Type I acceptor cannot be properly accommodated by 11639FucT because of the flipped GlcNAc moiety.

In conclusion, *H. pylori* α-(1,3/1,4)-FucTs and their mammalian counterparts seem to share striking functional similarities despite a very low level of sequence homology. The functional similarities that have been shown previously with respect to domain architecture and the region and residues that discriminate Type I from Type II acceptors (24, 44) have now been extended to include the minimal catalytic domain, kinetic parameters, and key polar groups of acceptors that are essential for enzyme recognition. Our data suggest that all α-(1,3/1,4)-FucTs very likely share a conserved mechanistic and structural basis for fucose transfer. This makes *H. pylori* α-(1,3/1,4)-FucTs an attractive model system for studying the entire FucT family, and the success in purifying soluble, stable, and active truncated forms of *H. pylori* α-(1,3/1,4)-FucTs allows structural studies to be pursued. In addition, *H. pylori* α-(1,3/1,4)-FucTs have great potential to be exploited in the enzymatic synthesis of fucosylated glycoconjugates. Such glycoconjugates have generated broad pharmaceutical interest for the prevention of bacterial and viral infections, the neutralization of toxins, and in immunotherapy for cancer (59–62).

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