Cloning and Characterization of the α(1,3/4) Fucosyltransferase of Helicobacter pylori*

(Received for publication, August 18, 1999, and in revised form, November 1, 1999)

David A. Rasko§§, Ge Wang‡‡, Monica M. Palcic***, and Diane E. Taylor‡ ‡‡

From the §Department of Medical Microbiology and Immunology and the ¶Department of Chemistry, University of Alberta, Edmonton, Alberta T6G 2H7, Canada

The gastric pathogen Helicobacter pylori can express the histo blood group antigens, which are on the surface of many human cells. Most H. pylori strains express the type II carbohydrates, Lewis X and Y, whereas a small population expresses the type I carbohydrates, Lewis A and B. The expression of Lewis A and Lewis X, as in the case of H. pylori strain UA948, requires the addition of fucose in α1,4 and α1,3 linkages to type I or type II carbohydrate backbones, respectively. This work describes the cloning and characterization of a single H. pylori fucosyltransferase (FucT) enzyme, which has the ability to transfer fucose to both of the aforementioned linkages in a manner similar to the human fucosyltransferase V (Fuc-TV). Two homologous copies of the fucT gene have been identified in each of the genomes sequenced. The characteristic adenosine and cytosine tracts in the amino terminus and repeated regions in the carboxyl terminus are present in the DNA encoding the two UA948fucT genes, but these genes also contain differences when compared with previously identified H. pylori fucTs. The UA948fucTa gene encodes an approximately 52-kDa protein containing 475 amino acids, whereas UA948fucTb does not encode a full-length FucT protein. In vitro, UA948FucTa appears to add fucose with a greater than 5-fold preference for type II chains but still retains significant activity using type I acceptors. The addition of the fucose to the type II carbohydrate acceptors, by UA948FucTa, does not appear to be affected by fucosylation at other sites on the carbohydrate acceptor, but the rate of fucose transfer is affected by terminal fucosylation of type I acceptors. Through mutational analysis we demonstrate that only FucTa is active in this H. pylori isolate and that inactivation of this enzyme eliminates expression of all Lewis antigens.

* The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1794 solely to indicate this fact.

§ Supported by A Doctoral Research Award from the Medical Research Council of Canada and an Alberta Heritage Foundation for Medical Research Studentship and Incentive Award.

¶ Supported by a Postdoctoral Fellowship from the Canadian Association of Gastroenterology and Astra Canada in association with an Medical Research Council-Pharmaceutical Manufacturer’s Association of Canada award as well as a fellowship from the Alberta Heritage Foundation for Medical Research.

** Supported by the Natural Sciences and Engineering Research Council of Canada.

†† Alberta Heritage Foundation for Medical Research Scientist. Supported from Canadian Bacterial Diseases Network as well as the National Cancer Institute of Canada with funds from the Terry Fox Run. To whom correspondence should be addressed: University of Alberta, Dept. of Medical Microbiology and Immunology, 1-28 Medical Sciences Bldg., Edmonton, AB T6G 2H7, Canada. Tel.: 780-492-4777; Fax: 780-492-7521; E-mail: diane.taylor@ualberta.ca.

† The abbreviations used are: LPS, lipopolysaccharide; PCR, polymerase chain reaction; ORF, open reading frame; CAT, chloramphenicol acetyltransferase.

‡ Monteiro, M. A., Appelmelk, B. J., Rasko, D. A., Hynes, S. O., McLean, L. L., Chan, K. H., St. Michael, F., Logan, S. M., O’Rourke, J., Lee, A., Moran, A. P., Taylor, D. E., and Perry, M. B. (2000) Eur. J. Biochem. 267, in press.

§ Rasko, D. A., Wilson, T. J. M., Zopf, D., and Taylor, D. E. (2000) J. Infect. Dis., in press.
enzyme substrate specificity (differential fucose transfer to type I or type II carbohydrate acceptors) (12, 21, 22). A consensus amino acid sequence for the α(1,3) FucT enzymes (11, 23, 24) has also been identified.

Two highly homologous copies of the α(1,3) fucT have been identified in both of the H. pylori genomes sequenced to date (25, 26). It is thought that the fucT genes are controlled by a slip strand repair mechanism at tracts of cytosines and adenines in the 5' end of the gene (27). In previous studies a fucT gene from NCTC11637 and a fucT gene from NCTC11639 have been cloned, and in vitro characterization of the FucT enzymes did not demonstrate α(1,4) FucT activity (28, 29). This is consistent with the observation that the lipopolysaccharide of these strains does not contain any type I Lewis antigens (30).

We have previously identified an H. pylori strain, UA948 that expresses both type I (Leα) and type II (Leβ) carbohydrate structures simultaneously (1). For H. pylori to produce the Lewis structures (Leα and Leβ) in the LPS O-chain, as for the production of Lewis antigens by human cells, there is a requirement for the addition of fucose to both α(1,3) and α(1,4) linkages. Thus it is expected that UA948 contains both α(1,3) and α(1,4) FucT activities (Fig. 1).

We have examined the FucTs from the H. pylori strain UA948 in an attempt to isolate the α(1,4) FucT activity. In this study we demonstrate that a single H. pylori FucT enzyme from UA948 contains both α(1,3) and α(1,4) FucT activity responsible for the production of both Leα and Leβ in the LPS O-side chain, while the other copy of the fucT gene does not encode a functional FucT enzyme. Comparisons of the nucleotide and amino acid sequences of the newly identified H. pylori α(1,3/4) FucT with the previously identified H. pylori α(1,3) FucTs allowed us to predict some of the domains of the enzyme that are potentially responsible for broadening the range of acceptors used by this enzyme.

**EXPERIMENTAL PROCEDURES**

**Bacterial Strains and Media—**H. pylori strains were cultured by standard methods described by Taylor et al. (31). Isolates from a frozen stock were thawed and plated out on BHI-YE agar plates (3.7% brain heart infusion, 0.5% yeast extract, 15 μg/ml of both vancomycin and amphotericin B, 5% of fetal bovine serum, 1.2% agar). These plates were incubated at 37°C under microaerobic conditions for 2–4 days. Positive H. pylori cultures were confirmed by urease test and microscopy. Transforms containing the chloramphenicol acetyltransferase gene (32) inserted into the fucT genes were isolated as described previously (28) and cultured on BHI-YEA plates as described above containing 50 μg/ml chloramphenicol (Sigma-Aldrich). *Escherichia coli* DH10β were grown on Luria broth agar plates containing 100 μg/ml ampicillin and/or 50 μg/ml chloramphenicol or 50 μg/ml kanamycin depending on the resistance markers present on the plasmids within the cells.

**DNA Manipulation Techniques—**Standard DNA manipulation techniques including the isolation, transformation, and restriction enzyme digestion analysis of plasmid DNA were detailed by Sambrook et al. (33). Both strands of the appropriate PCR fragments were sequenced using the Thermosequenase sequencing kit according to the manufacturer’s instructions. Sequence analyses were performed with the BLAST program from the National Center of Biotechnology Information (Bethesda, MD). The Wisconsin Package (version 9.0) of the Genetics Computer Group (Madison, WI) was used for the editing and alignment of sequences.

**Cloning and Overexpression of the H. pylori fucT Genes—**The primers used for amplification and cloning of the fucTs are as follows. To clone fucTa DAVE55 5’-gggatatcgccctggaattacctacctttctgc-3’ (positions 398988–399018) and DAVE56 5’-gggatatcgccctggaattacctacctttctgc-3’ (positions 399877–399898) were used. To clone fucTb DAVE53 5’-gggatatcgccctggaattacctacctttctgc-3’ (positions 698868–698892) and DAVE54 5’-gggatatcgccctggaattacctacctttctgc-3’ (positions 699638–699655) were used. The capital letters denote sequences derived directly from the published sequence from the strain 26695 (25), whereas the lowercase letters of the primers denote the restriction endonuclease sites used to facilitate cloning. PCR was performed as described previously (28) producing fragments of 1769 and 1774 nucleotides for the UA948/fucTa and UA948/fucTb fragments, respectively. Restriction with EcoRI and BamHI allowed cloning into a similarly digested pBluescript II KS+. The respective clones containing the H. pylori fucTa or fucTb were screened by the primers described above. The proposed coding region of the UA948/fucTa and UA948/fucTb were placed under the control of the T7 promoter.

Recombinant plasmids pB948fucTa and pB948fucTb were introduced by electroporation into *E. coli* K38 containing the plasmid pGPI-2 (which encodes a heat-inducible T7 RNA polymerase) (34). The proteins encoded by the recombinant plasmids were expressed as follows. *E. coli* K38(pGPI-2) harboring the plasmids containing the recombinant UA948/fucTa or UA948/fucTb plasmids were grown in 20 ml of liquid LB medium with appropriate antibiotics (kanamycin and ampicillin) at 30°C to an optical density of 0.5–0.7 at 600 nm. After collection by centrifugation, the cells were washed once in M9 medium, and resuspended in 5 ml of supplemented M9 medium and further incubated for 1 h at 30°C. To induce the expression of the recombinant gene, the culture was shifted to 42°C by adding 5 ml of 55°C supplemented M9 medium and incubated for a further 15 min at 42°C. Rifampicin was added to a final concentration of 200 μg/ml, and incu-
bation continued for 30 min. An aliquot was removed and incubated a further 30 min with [35S]methionine (50 nCi, Mallard Scientific Company Ltd., Guelph, ON, Canada), after which the cells were harvested by centrifugation and resuspended in sample buffer and subjected to SDS-polyacrylamide gel electrophoresis as described previously (28). The remaining culture was prepared for enzyme assay by methods previously described (28).

Fucosyltransferase Assay—The FucT assays were performed as described previously (35) with some modification. Reactions were conducted at 37 °C for 20 min in a 20-μl volume containing 1.8 mM acceptor, 50 μM GDP-fucose, 60,000 dpm GDP-3H,fucose (American Radiolabeled Chemicals Inc., St. Louis, MO), 20 mM HEPES buffer (pH 7.0), 10 mM MgCl2, 0.1 mM NaCl, 35 mM MgCl2, 1 mM ATP, 5 mg/ml bovine serum albumin, and 9.0 μl of the enzyme preparation. Acceptors used in this study were: Type I (β Gal 1–3 β GlcNAc-O-(CH2)8CO2CH3), H Type I (αFuc-1–2 β Gal 1–3 β GlcNAc-O-(CH2)8CO2CH3), H Type II (αFuc-1–2 β Gal 1–4 β GlcNAc-O-(CH2)8CO2CH3), and H Type II (αFuc-1–2 β Gal 1–4 β GlcNAc-O-(CH2)8CO2CH3). These acceptors were kindly provided by Dr. O. Hinds- gaul. For calculation of the specific activity of the enzyme (milli-units per milligram of protein), protein concentrations of the cell extracts were determined with a BCA protein assay kit (Pierce) using bovine serum albumin as a standard according to the supplier’s instructions.

Inactivation of the UA948fucTa—A chromaphenicol cassette inserted at a unique XmnI site in the fucT gene and the resultant construct (28) was used for transformation. Natural transformation of UA948 was accomplished by the method of Ge and Taylor (36). Briefly, a 48-h culture from frozen stock was restreaked and grown for 5 h on a BHI-YEA plate, 5 μg of DNA containing the fucT::CAT was added to the growth. After a further incubation for 20 h at 37 °C, the culture was plated onto BHI-YEA plate containing 50 μg/ml of chloramphenicol. Cultures were grown from single colonies, and the genomic DNA was extracted according to Ge and Taylor (36). Insertion of the CAT cassette in the fucT gene on the chromosome was confirmed by PCR with the previously described primers, which were specific to each copy of the fucT gene. PCR products were subjected to electrophoresis on a 1% agarose gel and photographed under UV light.

Enzyme-linked Immunosorbent Assay for Lewis Antigens—The conditions for the enzyme-linked immunosorbent assay were previously described (1, 37). The primary antibodies used were anti-Lewis A (MAb BG-7, clone P12), anti-Lewis B (MAb BG-6, clone T218), anti-Lewis X (MAb BG-7, clone P12), and anti-Lewis Y (MAb BG-8, clone F3) from Signet Laboratories Inc. (Dedham, MA). These primary antibodies were diluted 1:100, whereas the secondary antibody, goat anti-mouse IgG + IgM conjugated to horseradish peroxidase (Biocan number 115 035 068, Mississauga, ON, Canada), was diluted 1:2000. Absorbance values were recorded at 405 nm using a Titretek Multiscan MC microtitre plate reader. Absorbance values are an average of triplicate wells with blanks subtracted. Values below 0.1 absorbance units were considered negative.

Analysis of LPS by Acrylamide Gel Electrophoresis and Immunoblotting—Whole cell extracts of the H. pylori strains were treated with proteinase K, processed, and subjected to electrophoresis as described previously (1). These gels were stained with either zine imidazole, according to the method of Hardy et al. (38), or transferred to nitrocellulose membrane (Micron Separations Inc., Westboro, MA; pore size, 0.22 μm) according to the method described by Towbin et al. (39). Nitrocellulose membranes, with transferred LPS, were probed with the primary and secondary antibodies described above (anti-Lewis structure from Signet Laboratories Inc., antibodies 1:500 dilution and goat anti-mouse conjugated to horse- radish peroxidase diluted 1:2000, respectively). Blots were developed using an enhanced chemiluminescence kit (Amersham Pharmacia Biotech) according to the manufacturer’s specifications, and images were visualized on BioMax BM film (Eastman Kodak Co., Rochester, NY).

RESULTS

Features of the α(1,3/4) fucT Gene—PCR and subsequent sequence analysis of the inserts of the pB948fucTa and pB948fucTb demonstrated a significant difference in the size of the UA948fucTb gene compared with the predicted size from strain 26695. The insert in pB948fucTb contained 1774 base pairs, whereas the predicted size is 2030 base pairs. The main reason for the difference in the size is the absence of a number of repeated sequences in UA948fucTb that exist in the 3’ region of 26695fucTb. On the other hand, the PCR product of UA948fucTa is 1769 base pairs long, which is similar to the predicted size of 1799 base pairs. The nucleotide sequence of UA948fucTa is 85.5% identical to the fucTa of 26695, whereas the UA948fucTb is 87.6% identical when compared with the fucTb gene in 26695. Like in other H. pylori fucT genes identified (25, 26, 28, 29), characteristic cysteine and adenosine tracts exist in the 5’ end of the UA948fucT genes (Table I).

The protein translation of the sequenced PCR fragments revealed that the UA948fucTa gene encodes an open reading frame (ORF) of 475 amino acids with a predicted molecular mass of 55.9 kDa, whereas UA948fucTb does not encode an ORF containing a full-length FucT protein. An artificial increase or decrease in the cysteine and/or adenosine tract of the UA948fucTb gene by any small number of nucleotides does not provide a full-length fucT ORF from this gene, eliminating the possibility that the lack of functional protein is caused by slip strand mispairing. A similar modification was discovered to be necessary for the production of full-length protein by NCTC11637 (29) and J99fucTa (JHP1002) (26). An ORF with homology to the identified FucTs does exist in the UA948fucTb insert, but it is truncated.

A comparison of the UA948fucTa amino acid sequence with previously identified H. pylori FucTs demonstrates a high level of homology (Fig. 2). Overall, a greater than 70% amino acid identity was noted between the identified H. pylori FucTs. However, this homology is largely confined to an internal approximately 270 amino acids, which demonstrates a 82.2% identity among all H. pylori FucTs. The highly conserved internal 270 amino acids of H. pylori FucTs contains the hypothetical α(1,3) catalytic domains identified in eukaryotic FucTs by sequence alignments (23, 24) (Fig. 2A, domains I and II). All H. pylori FucTs contain a conserved domain I with a low level identity with only 2 of 19 amino acids being conserved, whereas a much greater level of identity is present in domain II, with 12 of 23 amino acids being conserved (Fig. 2A). The human FucTs (H-FucT) have a conserved carboxyl terminal containing the catalytic domain and an amino terminus containing the transmembrane and variable regions, whereas the H. pylori FucT (Hp-FucT) have an internally conserved region containing the catalytic domain, and both the amino and carboxyl termini are variable (Fig. 2B).

Among H. pylori FucTs the first 82 amino acids exhibit only 32.0% identity. Although it is difficult to calculate the degree of identity over the carboxyl-terminal portion of these proteins

| Strain and fucT copy | Nucleotide | Amino acid |
|----------------------|------------|------------|
| Strain and fucT copy | Adenosine repeat | Cystine repeat | Number of repeats | Sequence of repeat |
| 26695A | 6 | 13 | 2 | DDLRVNY |
| 26695B | 9 | 13 | 10 | DDLRVNY |
| J99A | 9 | 13 | 4 | DDLRVNY |
| J99B | 3 | 5 | 7 | DDLRVNY |
| NCTC11639 | 6 | 10 | 10 | DDLR(V/I)NY * |
| NCTC11637 | 6 | 9 | 7 | D/N/D/L/R/V/I/NY |
| UA948A | 3 | 5 | 8 | DDLRVNY/D/DLLRDH/R/ |
| UA948B* | 9 | 9 | NA* | NA* |

* Artificially adjusted by 1 nucleotide to produce a full-length FucT protein.

* Repeats 3, 5, and 8 contain an isoleucine instead of a valine at position five of the repeat.

* The first two repeat sequences contain the sequence DNLRVNY, the following two repeats contain the consensus repeat sequence DDLRVY, and the final three repeats contain the sequence DDLRNY.

* Repeats 1 and 8 contain the sequence DDLRRDH, whereas Repeat 7 has the sequence DDLRRDR.

* NA, not applicable because no protein is produced. Adjustment of either polynucleotide repeat region does not allow the production of full-length protein.
(final approximately 100 amino acids), as in the carboxyl terminus of all identified Hpo(1,3) FucTs (25, 26, 28, 29) there exists a variable number and sequence of a 7-amino acid repeat (heptad repeat) (Table I and Fig. 2). These heptad repeats are thought to function as a leucine zipper in dimerization, which may be essential for function (28, 29). In addition, all identified Hpylori a(1,3) FucTs identified the heptad repeat consists of the amino acids DDLRDN, UA948FucTa contains five internal repeats that are of this consensus heptad sequence, whereas the remaining three repeats show divergence. The two heptads that border the repeat region contain the amino acid sequence DDLRRD, whereas the second last heptad contains the amino acid sequence DDLRRDR (Table I and Fig. 2A). Following the heptad repeats there is also a 15-amino acid addition at the carboxyl terminus of the UA948FucTa protein that does not show homology with any protein or motif presently in the databases.

**Protein Expression**—Both the UA948FucTa and UA948FucTb were directionally cloned into pBluescript KS+ under the control of the T7 promoter. Expression of the protein was accomplished by utilization of the strain K38 containing a heat-inducible T7 polymerase on the plasmid pGP1-2 (34). Under expression conditions pBUA948FucTa-containing cells expressed a protein of approximately 52 kDa, which is in close agreement with the predicted size of the protein based on the amino acid sequence.

---

**Fig. 2.** A, comparison of the amino acid sequences of the known Hpylori FucTs. J99A, JHP1002 (AAD06573); J99B, JHP0596 (AAD06169); 26695A, HP0379 (AAD07447); 26695B, HP0651 (AAD07710); NCTC11639, the one copy identified (AAB81031); NCTC11637, the one copy identified (AAB93985); UA948A, FucTa of H. pylori UA948 (AF194963). Numbers in parentheses above refer to the GenBank accession number. Asterisks indicate amino acid identity, double dots indicate a conserved amino acid substitution, and a blank space indicates a nonconservative amino acid substitution. The conserved catalytic domains are designated by a line on top of the sequence, and the cross-species conserved amino acids within this region are in bold type. Amino acid sequences were aligned by CLUSTALW alignment. B, a diagrammatic alignment of human FucTs with Hpylori FucTs. In both cases N denotes the amino terminus and C is the carboxyl terminus, whereas CAT represents the catalytic domains of the FucT enzymes. HV is the hypervariable region identified in the human FucTs, which contain mutations responsible for the alteration of enzyme characteristics. TM is the transmembrane region present only in the human FucTs. V is the variable regions identified in the Hpylori FucTs.
effects of the mutation of UA948fucTa on the mobility of the LPS and the Lewis antigens in the LPS (Fig. 5 and Table III). The LPS from UA948 contains only Lewis A structures, whereas the wild type LPS contains both Lewis A and X structures in a 50:50 ratio. The mutation of UA948fucTa has a dramatic effect on the mobility of the LPS and the expression of Lewis antigens in the LPS (Fig. 5 and Table III). The LPS from UA948fucTa migrates more slowly through the SDS-polyacrylamide gel electrophoresis gel, as indicated by the arrow in Fig. 5A, when compared with the wild type LPS. The band is more diffuse and thus less intense than the wild type LPS. The expression of the Lewis antigens has also been eliminated in UA948fucTa, indicating that the only functional FucT in H. pylori UA948 is FucTa.

**DISCUSSION**

The single functional H. pylori FucT enzyme identified in this study is a novel enzyme in both activity and specificity when compared with the previously characterized H. pylori FucTs (28, 29). Both previously identified FucTs almost exclu-
sis of the UA948 and UA948fucTa.

...some H. pylori difucosylated antigens by ...necessary to definitively identify the pathway for the synthesis of that the UA948fucTa allows the authors to believe that the production of the difucosylated Type II, as an acceptor to produce Lewis Y, thus leading the enzyme-linked immunosorbent assay values of UA948 wild type versus UA948fucTa−

| Lewis antigen | UA948 | UA948fucTa− |
|---------------|-------|-------------|
| Lewis A⁺      | 3.34 ± 0.04 | 0.032 ± 0.002 |
| Lewis X⁺      | 0.78 ± 0.06 | 0.003 ± 0.001 |

Antigens screened for by using method previously described by Monteiro et al. (1).

sively transferred fucose to type II carbohydrate acceptors (28, 29). Martin et al. (29) did note some enzyme activity with an elongated type I carbohydrate acceptor, but no activity was noted with the minimal type I disaccharide lacto-N-biose (Type I chain) used in this study. The UA948FucTa enzyme identified in this work can add fucose to both type I and II carbohydrate acceptors (Table II), representing the first α,1,3/4 FucT to be identified in H. pylori. There is a greater than 5-fold preference for the type II carbohydrate acceptors over type I carbohydrate acceptors, which is more similar to the enzyme characteristics exhibited by the human Fuc-TV than any other enzyme (40). The human Fuc-TV enzyme shows a slight preference for type II acceptors but still retains significant activity on type I carbohydrate acceptors.

It was also noted in the study by Martin et al. (29) that the α,1,3FucT isolated could not use the type II carbohydrate, H Type II, as an acceptor to produce Lewis Y, thus leading the authors to believe that the production of the difucosylated Lewis antigens, Leα and Leβ, may be routed through a subterminal monofucosylation by an α,1,3 or α,1,3/4 FucT followed by the terminal fucosylation by an α,1,2 FucT (41) (Fig. 1, A and B, right-hand pathway). It is apparent from the data presented in this study that this is not true for all H. pylori α (1,3)FucTs, because UA948FucTa has the ability to add fucose in a subterminal position on the GlcNAc of H Type II very efficiently, as well as to H Type I with reduced efficiency produce a difucosylated antigens (Fig. 1, A and B, left-hand pathway). Our recent observations show that the α,1,2 FucT of some H. pylori isolates can use both the subterminally fucosylated (Lewis A or Lewis X) as well as the unfucosylated carbohydrate chains (Type I and LacNac) as acceptors. More work with both the α,1,2 FucT and the α,1,3/4 FucT is necessary to definitively identify the pathway for the synthesis of difucosylated antigens by H. pylori.

Although two homologous copies of the fucT gene exist within the H. pylori genome of most H. pylori strains, only one appears to be active in H. pylori UA948. Interestingly, even though the UA948fucTb gene is inactive, with respect to FucT activity, no insertions of the CAT cassette into this gene could be obtained. We have also observed this phenomenon in other H. pylori isolates while attempting to make a double mutant in which both of the fucT genes are insertionally inactivated. This suggests that UA948fucTb may encode an unknown essential gene product or the genes flanking UA948fucTb are essential and may be transcriptionally or translationally linked. Upstream of UA948fucTb is the gene encoding a cytochrome C biogenesis protein, which terminates only 13 nucleotides upstream of the proposed start codon of the fucTb ORF in both 26695 (25) and J99 (26). It is possible that these genes are coordinately transcribed and that insertion of the CAT cassette into the fucTb gene affects the synthesis of the cytochrome C biogenesis protein, which would be deleterious to the cell, whereas there are amino acid biosynthetic genes in both the upstream and downstream positions of UA948fucTb. Probably the inactivation of these genes do not pose a strong selective pressure on the H. pylori, considering the richness of the media used for culture.

The LPS of UA948fucTa− no longer contains any of the complex, fucose-containing, Lewis antigens that are present in the wild type UA948 strain, even though only a single fucT gene has been inactivated (Fig. 5 and Table III). This further supports the conclusion that only one copy of the fucT gene is active in this H. pylori isolate. The inactivation of the only fucT gene in this H. pylori isolate provides an opportunity to determine what role, if any, the formation of complexed fucose containing carbohydrates in the LPS plays in the infectious process of H. pylori.

It has been noted in the identification of the key amino acids involved in the specificity of the human FucTs that single amino acid changes can change or eliminate the expression of the FucT activity (12, 17–22). Because of the low level homology between the prokaryotic and eukaryotic α,1,3 FucTs, it is difficult to use the amino acid sequence of the human FucTs to predict which amino acid changes may be responsible for the alteration of acceptor specificity of H. pylori FucTs, but the human system may provide clues as to the identification of the region responsible for this activity in the H. pylori FucTs. Breton et al. (23, 24) identified two conserved motifs within the catalytic domain that is identical in all of the human α,1,3 FucT enzymes regardless of the acceptor specificity, and these

---

5 D. A. Rasko, G. Wang, M. M. Pulcic, and D. E. Taylor, unpublished data.

![Image](http://www.jbc.org/content/279/29/4993/F5.large.jpg)

**FIG. 5.** LPS and immunoblot analysis of the UA948 and UA948fucTa−. In all panels lane 1 shows a proteinase K-treated sample of UA948, and lane 2 shows a similarly treated sample of UA948fucTa−. A is a zinc-imidazole-stained polyacrylamide gel showing the change in mobility of the LPS from the UA948fucTa− isolate as is indicated by the arrow. B and C are immunoblots probed with anti-Lewis A and anti-Lewis X, respectively. Both panels demonstrate that the UA948fucTa− isolate no longer expresses Lewis antigens.
domains are present in the α(1,3) FucT of other species (11). By sequence analysis these two motifs have been localized in the \emph{H. pylori} α(1,3) FucTs (Fig. 2A, domains I and II), and they are also present in the α(1,3/4) FucT of \emph{H. pylori}. The \emph{H. pylori} FucTs have a high level of amino acid identity (−82%) in the internal 270 amino acids of the FucT proteins corresponding to the proposed catalytic domain. In the human FucTs mutations in this highly conserved region generally correspond to the inactivation of the FucT activity of the protein (18–20, 42).

Obvious differences were observed when the UA948FucTa was compared with the previously identified \emph{H. pylori} FucTs. Firstly, there was a low level of homology (−30%) in the amino-terminal 80 amino acids of \emph{H. pylori} FucTs, which in the human FucTs is the location of the transmembrane and hypervariable region (Fig. 2B). The hypervariable region is the location of essential amino acids responsible for the determination of the enzyme acceptor specificity (11, 12). All of the \emph{H. pylori} FucTs examined by our group appear to have differing rates of the enzyme acceptor specificity (11, 12). All of the \emph{H. pylori} (1,3) FucTs (Fig. 2). The first four amino acids of the UA948FucTa heptad repeat are conserved, DDLR, but the final three amino acids are divergent. The UA948FucTa heptad repeats still contain the leucine moieties in the appropriate spacing to continue acting as a leucine zipper. It is clear that although variable, there is some conservation of physical attributes of the variable amino acids in the repeat region because the net charge of these three amino acids is relatively conserved. It is unclear at this time whether the nonhomologous flanking heptad repeats are involved in the broadening of the acceptor range to include the type I carbohydrate acceptors or whether these regions are responsible only for the hypothesized function of dimerization (28, 29). Finally, there is also a carboxy-terminal 15-amino acid addition in UA948FucTa, which is not present in the other \emph{H. pylori} α(1,3) FucTs and does not share any homology with any identified sequence. It was noted in Ge et al. (28) that a carboxy-terminal 15-amino acid truncation eliminated all FucT enzyme activity, proving that this region is essential for enzyme activity.

We have demonstrated the existence of an α(1,3/4)FucT from \emph{H. pylori} that is responsible for the production of both Leα and Leβ (Fig. 1). This enzyme exhibits significant sequence divergence at both the nucleotide and amino acid level from previously identified \emph{H. pylori} FucTs. The regions of variability will need further investigation to determine their role, if any, in the activity and expanded acceptor range of UA948FucTa. It will require a careful molecular study, identification of more \emph{H. pylori} α(1,3) FucTs, and domain swapping experiments similar to those performed with the human FucTs to determine exactly which of the changes are responsible for the broader acceptor range of UA948FucTa.

Acknowledgments—We thank P. Boulot and Nora Chan for assistance with the FucT assays.

REFERENCES

1. Monteiro, M. A., Chan, K. H. N., Rasko, D. A., Taylor, D. E., Zheng, P. Y., Appelmelk, B. J., Wirth, H.-P., Yang, M., Blaser, J. M., Hynes, S. O., Moran, A. P., and Perry, M. B. (1998) J. Biol. Chem. 273, 11533–11543
2. Appelmelk, B. J., Simoons-Smit, I., Negrini, R., Moran, A. P., Aspinall, G. O., Forte, J. G., Devries, T., Quan, H., Verboom, T., Maaskant, J. J., Ghia, P., Kuijpers, E. J., Bloemenda, E., Tadema, T. M., Townsend, R. R., Tyagarajan, K., Crothers, J. M., Monteiro, M. A., Savio, A., and Degraaff, J. (1996) Infect. Immun. 64, 2031–2040
3. Taylor, D. E., Rasko, D. A., Sherburne, R., Ho, C., and Jewel, L. D. (1998) Gastroenterology 115, 1113–1122
4. Negrini, R., Savio, A., Poiesi, C., Appelmelk, B. J., Buffoli, F., Paterlini, A., Cesari, S., Graffeo, M., Vaira, D., and Franzin, G. (1996) Gastroenterology 111, 655–665
5. Amano, K., Hayashi, S., Kubota, T., Fijii, N., and Yokata, S. (1997) Clin. Diag. Lab. Immunol. 4, 540–546
6. Claeyts, D., Fallier, G., Appelmelk, B. J., Negrini, R., and Kirchner, T. (1998) Gastroenterology 115, 340–347
7. Simoons-Smit, I. M., Appelmelk, B. J., Verboom, T., Negrini, R., Penner, J. L., Aspinall, G. O., Moran, A. P., She, F. F., Shi, B. S., Rudnica, W. S., Savio, A., and De Graaff, J. (1996) J. Clin. Microbiol. 34, 2196–2200
8. Costache, M., Cailleau, A., Fernandez-Mateos, P., Oriol, R., and Mollicone, R. (1997) Transfus. Clin. Biol. 4, 367–382
9. Henry, S., Oriol, R., and Samuelsson, B. (1995) Vox Sang. 69, 166–182
10. Kukawka-Latallo, J., Larsen, R. D., Nair, R. P., and Lowe, J. B. (1996) Gene 194, 1288–1303
11. Oriol, R., Mollicone, R., Cailleau, A., Balanzino, L., and Breton, C. (1999) Glyobiology 9, 323–334
12. Dupuy, P., Petit, F.-M., Mollicone, R., Oriol, R., Julien, R., and Matath, A. (1999) J. Biol. Chem. 274, 12257–12262
13. Weston, B. W., Nair, R. P., Larsen, R. D., and Lowe, J. B. (1992) J. Biol. Chem. 267, 4152–4160
14. de Vries, T., Sruka, C. A., Palic, M. M., Swiedler, S. J., van den Eijnden, D., and Macher, B. A. (1995) J. Biol. Chem. 270, 8712–8722
15. Costa, J., Grabenhorst, E., Nimtz, M., and Conrad, H. S. (1997) J. Biol. Chem. 272, 11613–11621
16. Ge, Z., and Taylor, D. E. (1992) in \emph{Carbohydrate Structures in Antigens and \emph{H. pylori} (1,3) FucTs \emph{H. pylori} FucTs. This region contains the heptad repeats, that is the proposed leucine zipper region, UA948FucTa exhibits significant amino acid sequence variability when compared with previously identified \emph{H. pylori} α(1,3) FucTs (Fig. 2). The first four amino acids of the UA948FucTa heptad repeat are conserved, DDLR, but the final three amino acids are divergent. The UA948FucTa heptad repeats still contain the leucine moieties in the appropriate spacing to continue acting as a leucine zipper. It is clear that although variable, there is some conservation of physical attributes of the variable amino acids in the repeat region because the net charge of these three amino acids is relatively conserved. It is unclear at this time whether the nonhomologous flanking heptad repeats are involved in the broadening of the acceptor range to include the type I carbohydrate acceptors or whether these regions are responsible only for the hypothesized function of dimerization (28, 29). Finally, there is also a carboxy-terminal 15-amino acid addition in UA948FucTa, which is not present in the other \emph{H. pylori} α(1,3) FucTs and does not share any homology with any identified sequence. It was noted in Ge et al. (28) that a carboxy-terminal 15-amino acid truncation eliminated all FucT enzyme activity, proving that this region is essential for enzyme activity.

We have demonstrated the existence of an α(1,3/4)FucT from \emph{H. pylori} that is responsible for the production of both Leα and Leβ (Fig. 1). This enzyme exhibits significant sequence divergence at both the nucleotide and amino acid level from previously identified \emph{H. pylori} FucTs. The regions of variability will need further investigation to determine their role, if any, in the activity and expanded acceptor range of UA948FucTa. It will require a careful molecular study, identification of more \emph{H. pylori} α(1,3) FucTs, and domain swapping experiments similar to those performed with the human FucTs to determine exactly which of the changes are responsible for the broader acceptor range of UA948FucTa.
Cloning and Characterization of the α(1,3/4) Fucosyltransferase of *Helicobacter pylori*

David A. Rasko, Ge Wang, Monica M. Palcic and Diane E. Taylor

*J. Biol. Chem.* 2000, 275:4988-4994.
doi: 10.1074/jbc.275.7.4988

Access the most updated version of this article at [http://www.jbc.org/content/275/7/4988](http://www.jbc.org/content/275/7/4988)

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 40 references, 20 of which can be accessed free at [http://www.jbc.org/content/275/7/4988.full.html#ref-list-1](http://www.jbc.org/content/275/7/4988.full.html#ref-list-1)