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The ruminant parasite *Haemonchus contortus* expresses an α1,3-fucosyltransferase capable of synthesizing the Lewis x and sialyl Lewis x antigens

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Glycoproteins from the ruminant helminthic parasite *Haemonchus contortus* react with *Lotus tetragonolobus* agglutinin and *Wisteria floribunda* agglutinin, which are plant lectins that recognize α1,3-fucosylated GlcNAc and terminal β-GalNAc residues, respectively. However, parasite glycoconjugates are not reactive with *Ricinus communis* agglutinin, which binds to terminal β-Gal, and the glycoconjugates lack the Lewis x (Le^x) antigen or other related fucose-containing antigens, such as sialylated Le^x, Le^a, Le^b Le^y, or H-type 1. Direct assays of parasite extracts demonstrate the presence of an α1,3-fucosyltransferase (α1,3FT) and β1,4-N-acetylgalactosaminyltransferase (β1,4GalNAcT), but not β1,4-galactosyltransferase. The *H. contortus* α1,3FT can fucosylate GlcNAc residues in both lacto-N-neotetraose (LNnT) Galα1→4GlcNAcβ1→3Galβ1→4Glc to form lacto-N-fucopentaose III Galβ1→4[Fucα1→3]GlcNAcβ1→3Galβ1→4Glc, which contains the Le^x antigen, and the acceptor lacdiNAc (LDN) GalNAcβ1→4GlcNAc to form GalNAcβ1→4[Fucα1→3]GlcNAc. The α1,3FT activity towards LNnT is dependent on time, protein, and GDP-Fuc concentration with a K_m 50 μM and a V_{max} of 10.8 nmol-mg^{-1} h^{-1}. The enzyme is unusually resistant to inhibition by the sulfhydryl-modifying reagent N-ethylmaleimide. The α1,3FT acts best with type-2 glycan acceptors (Galβ1→4GlcNAcβ1-R) and can use both sialylated and non-sialylated acceptors. Thus, although *in vitro* the *H. contortus* α1,3FT can synthesize the Le^x antigen, *in vivo* the enzyme may instead participate in synthesis of fucosylated LDN or related structures, as found in other helminths.

***Keywords*: *Haemonchus contortus*; α1,3-fucosyltransferase; Le^x antigen; β1,4-galactosyltransferase; β1,4-N-acetylgalactosaminyltransferase**

***Abbreviations*: NDV, New Castle Disease; WFA, *Wisteria floribunda* agglutinin; LTA, *Lotus tetragonolobus* agglutinin; NEM, N-ethylmaleimide; Le^a, Lewis a antigen; Le^x, Lewis x antigen; sLe^x, sialyl Lewis x antigen; Le^y, Lewis y antigen; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; PBS, phosphate buffered saline; TTBS, Tween-20/Tris buffered saline; EDTA, ethylenediaminetetraacetic acid; PMSF, phenylmethylsulfonyl fluoride; BCA, bicinchoninic acid**

Introduction

Haemonchus contortus is a parasitic nematode of small ruminants and is one of the most important nematodes infecting goats and sheep. The parasite feeds on host blood and causes anemia, eventually leading to the morbidity and mortality associated with this infection. Protective immunity to *H. contortus* can be induced by using carbohydrate-containing antigens [1–3]. In addition, a substantial part of the host immune serum antibody response in sheep immunized with H11 gut antigen is directed against periodate-sensitive, presumably carbohydrate-containing, epitopes [4]. Other studies have also found that a substantial part of the host-immune response to *H. contortus* antigens is to periodate-sensitive epitopes [5]. The role of parasite carbohydrate epitopes in immunity remains to be determined. Although little is known about the structures of the carbohydrate antigens in *H. contortus*, recent studies reveal unusual structures of some fucose-containing N-glycans in *H. contortus* glycoproteins and purified H11, which is a gut-associated antigen [6]. It is hoped that increased knowledge of these carbohydrate-containing antigens may lead to a better understanding of the host-parasite interactions and the role these moieties might have in relation to protective immunity.

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In other helminthic parasites, such as *Schistosoma mansoni*, surface carbohydrates in glycoproteins and glycolipids are major antigenic determinants and fucose is a major component of the antigens [7–15]. Such fucosylated structures may be important in host-parasite interactions in schistosomiasis [16, 17]. In adult *S. mansoni* fucose is present in glycoproteins in two types of structures, which are designated the Lewis x (Le^x) antigen Galβ1 → 4[Fucα1 → 3] GlcNAc-R and the related structure built on the lacdiNAc-type (LDN) motif GalNAcβ1 → 4[Fucα1 → 3]GlcNAc [8, 9, 17]. This latter structure, but not Le^x, is also present in microfilariae of *Dirofilaria immitis* [18]. The common occurrence of α1,3-linked fucose and terminal β1,4-linked GalNAc in flatworms and roundworms led us to examine whether these structures might also be present in glycoproteins of *H. contortus*.

We report here that detergent extracts of *H. contortus* contain high levels of an α1,3FT activity and that this activity has properties comparable to the α1,3FT activity found in adult schistosomes [19] and the human myeloid enzyme FT-IV [20–23]. Interestingly, the glycoconjugates of *H. contortus* do not contain detectable amounts of the Le^x determinant. Glycoproteins from *H. contortus* are bound by the plant lectins *Wisteria floribunda* agglutinin (WFA) and *Lotus tetragonolobus* agglutinin (LTA), which recognize terminal β-GalNAc and α1,3-fucosylated GlcNAc residues, respectively. Furthermore, β1,4 GalNAcT activity, but no β1,4 GalT activity is expressed by *H. contortus*. These results suggest that *H. contortus* glycoproteins may contain α1,3-fucosylated LDN-type structures, as is seen in *Dirofilaria immitis* and in some N-glycans from *S. mansoni*.

OLIGOSACCHARIDE STRUCTURES

LNT	Galβ1→3GlcNAcβ1→3Galβ1→4Glc
LNFPII	Galβ1→3(Fuc1→4)GlcNAcβ1→3Galβ1→4Glc
LNnT	Galβ1→4GlcNAcβ1→3Galβ1→4Glc
LNFPIII	Galβ1→4(Fuc1→3)GlcNAcβ1→3Galβ1→4Glc
LN	Galβ1→4GlcNAc
Le^x	Galβ1→4(Fuc1→3)GlcNAc
LacdiNAc (LDN)	GalNAcβ1→4GlcNAc
LDNF	GalNAcβ1→4(Fuc1→3)GlcNAc
2,3 sLN	NeuAcα2→3Galβ1→4GlcNAc
2,3 sLe^x	NeuAcα2→3Galβ1→4(Fuc1→3)GlcNAc
2,6 sLN	NeuAcα2→6Galβ1→4GlcNAc

Figure 1. Structures of glycans used in this study.

Materials and methods

Materials

Sodium cacodylate, $MnCl_2$, EDTA, ATP, L-fucose, NEM, GDP-Fuc, GlcNAc, LN, UDP-GalNAc, UDP-Gal, and QAE-Sephadex were purchased from Sigma Chemical Co. (St Louis, MO). LNT, LNnT, 2,3sLN, and 2,6sLN were obtained from V-Labs, Inc. (Covington, LA). The oligosaccharide acceptors and their abbreviations are listed in Figure 1. The lacdiNAc disaccharide (LDN) GalNAcβ1 → 4GlcNAc was prepared as previously described [24]. GDP-[^{3}H]-Fuc (7.1 Ci mmol^{-1}), UDP-[^{3}H]GalNAc (6.3 Ci mmol^{-1}), and UDP-[^{3}H]Gal (48.32 Ci mmol^{-1}) were purchased from NEN Research Products (Boston, MA). Triton X-100 was purchased from Bio-Rad Laboratories (Richmond, CA). *Streptomyces* α1,3/4 fucosidase (1mU ml^{-1}) was acquired from Pan Vera Corporation (Madison, WI). NDV-neuraminidase was purchased from Boehringer Mannheim (Indianapolis, IN). Biotinylated WFA and LTA were purchased from Vector Laboratories, Inc. (Burlingame, CA). The standards ^{3}H-LNFPIII, ^{3}H-Le^x Galβ1 → 4[^{3}H-Fucα1 → 3]GlcNAc and ^{3}H-LDNF GalNAcβ1 → 4[^{3}H-Fucα1 → 3]GlcNAc) were synthesized from LNnT, LN, and LDN acceptors, respectively, using extracts of COS7/FTIV cells as the source of α1,3FT and GDP-[^{3}H]-Fuc as the donor. The standards ^{3}H-LNFPII and ^{3}H-2,3-sialyl-Le^x were prepared from LNT and 2,3sLN, respectively, using extracts of COS7/FTIII cells as the source of α1,3FT and GDP-[^{3}H]-Fuc as the donor molecule. Reagents for SDS-PAGE and nitrocellulose membrane were purchased from Bio-Rad Laboratories (Hercules, CA). Streptavidin-peroxidase was obtained from Boehringer-Mannheim (Indianapolis, IN).

Antibodies

Monoclonal antibodies specific for Le^a, Le^b, Le^y and H-type-1 were purchased from Signet Laboratories, Inc. (Dedham, MA). Anti-Le^x mAb (Anti-CD15) and anti-sLe^x mAb (CSLEX-1) were obtained from Becton-Dickinson (San Jose, CA). Anti-mouse IgM and IgG-alkaline phosphatase conjugates were obtained from Sigma (St. Louis, MO). Anti-mouse IgM-peroxidase was purchased from Kirkegaard & Perry Laboratories (Gaithersburg, MD).

Preparation of *H. contortus* homogenates

H. contortus (Beltsville isolate) were maintained by passage through lambs, as described previously [1]. Adult parasites were isolated at necropsy and separated from abomasal debris by rinsing in ice-cold PBS. The parasites were stored at −80 °C until homogenized for enzyme assays. Frozen worms were thawed, washed three times in PBS (6.7 mM KH_2PO_4, 150 mM NaCl, pH 7.4) and suspended in 50 mM

cacodylate buffer, pH 7.0, containing the protease inhibitors, EDTA (37 μg ml^{-1}), leupeptin (0.5 μg ml^{-1}), pepstatin (0.7 μg ml^{-1}), PMSF (100 μM), and trypsin inhibitor (10 μg ml^{-1}). The parasite suspension was homogenized on ice with a Biohomogenizer (Model M 133/1281-0; Biospec Products, Inc., Bartlesville, OK) using three pulses of 10 sec each. The homogenate was subsequently subjected to sonication (three pulses of 10 sec each) on a Branson cell disrupter (Model 185) to effect complete disruption of the parasites. The homogenate was adjusted to 1% Triton X-100 and incubated on ice for 30 min to allow solubilization of proteins. The homogenate was centrifuged at 14,000 rpm, at 4 °C, for 10 min, and the supernatant fraction was collected. Protein concentration of the extract was determined by the BCA protein assay procedure (Pierce Co., Rockford, IL). The parasite extract was either used directly or stored as aliquots at −80 °C. Frozen extracts were thawed only once for use in enzyme assays.

Preparation of COS7/FTIII and COS7/FTIV extracts

As a source of α1,3FT activity, the recombinant human fucosyltransferases III and IV (FTIII and FTIV, respectively) were obtained from COS7 cells stably transfected with cDNAs encoding the respective genes. The transfected COS7 cells expressing these human fucosyltransferases, COS7/FTIII and COS7/FTIV, were shown to express high levels of enzyme activity [21, 23, 25]. The cells were maintained in DMEM containing 10% fetal bovine serum, 400 μg ml^{-1} G418, and supplemented with 2 mM glutamine. To prepare extracts, the cells were grown to 100% confluence, washed three times with PBS and detached from the culture plates by scraping with a rubber policeman in the presence of PBS. The cells were subsequently recovered by centrifugation at 1,000 rpm for 5 min. The cells were resuspended in an equal volume of 50 mM cacodylate buffer (pH 7.0), sonicated on ice (three pulses of ten seconds each in a Branson cell disrupter, Model 185), and adjusted to 1% Triton X-100. The protein content was determined using the BCA protein assay method. Cell extracts were used immediately in enzyme assays or stored as aliquots at −80 °C.

Fucosyltransferase assays

Fucosyltransferase assays were performed in 50 μl of 100 mM sodium cacodylate buffer, pH 7.0, containing 20 mM $MnCl_2$, 5 mM ATP, 15 mM fucose, 100,000 cpm GDP-[^{3}H]-Fuc (7.1 Ci mmol^{-1}) as the donor, 1.5 mM of either LNnT, LNT, 2,3sLN, 2,6sLN, LN or LDN as the acceptor, and extracts of either *H. contortus*, COS7/FTIII, or COS7/FTIV cells as the enzyme source. The reactions were incubated at 37 °C for different times and terminated by the addition of 450 μl water for reactions employing the neutral acceptors, LNnT, LNT, N-acetyllactosamine, and LDN, or 450 μl 2 mM pyridine/acetate buffer (pH 5.5) for reactions using 2,3sLN and 2,6sLN acceptors. Reaction products were isolated by ion exchange column chromatography on QAE-Sephadex as described below. Neutral products were not bound by the column and sialylated products were bound. Control experiments were performed in which no acceptor was added to the reaction. Radioactivity obtained from these control experiments represents free fucose generated by hydrolysis of the GDP-[^{3}H]-Fuc during the course of the reaction and was subtracted as background. The effect of N-ethylmaleimide (NEM) on the activity of fucosyltransferases was determined in reaction mixtures containing 0, 1, 5, 10, and 25 mM NEM. The reaction mixtures were preincubated on ice for 30 min followed by incubations at 37 °C for 3 h. The K_m of the *H. contortus* fucosyltransferase for GDP-Fuc was determined at a range of 1–200 μM (50 000 cpm nmol^{-1}) GDP-Fuc in reaction mixtures. All assays were performed in duplicate.

β1,4GalNAc- and β1,4Gal-transferase assays

β1,4Gal- and β1,4GalNAc-transferase assays were performed in a total volume of 50 μl containing 50 mM sodium cacodylate, pH 7.2, 20 mM $MnCl_2$, 5 mM ATP, 0.5% Triton X-100, 20 mM GlcNAc as the acceptor molecule, 25 μM UDP-[^{3}H]-Gal or UDP-[^{3}H]-GalNAc (50–60 cpm pmol^{-1}) as the sugar nucleotide donor, and varying amounts of *H. contortus* extract as the enzyme source. The reactions mixtures were incubated at 37 °C for 4 h and terminated by the addition of 450 μl water. The neutral disaccharide product, and free GalNAc or Gal generated during the course of the reaction, were isolated by ion exchange column chromatography on QAE-Sephadex and further fractionated by descending paper chromatography as described below. Control experiments were performed lacking acceptors.

Column chromatography

Ion exchange column chromatography was performed on 0.5 ml columns of QAE-Sephadex in 2 mM Tris-base, pH 8.0. Products of assays using LNnT, LNT, LN, and LDN were isolated by passage of reaction mixtures over columns of QAE-Sephadex equilibrated in water. The columns were washed with deionized water and 0.5 ml fractions were collected. The fractions were mixed with ScintiVerse and radioactivity was determined by liquid scintillation counting. Products of assays employing either 2,3sLN or 2,6sLN as acceptors were isolated by applying the reaction mixtures to 0.5 ml columns of QAE-Sephadex equilibrated with 2 mM pyridine/acetate buffer, pH 5.5. The columns were washed with 2.5 ml of 2 mM pyridine/acetate buffer to remove unbound, neutral material. The bound material containing charged products was eluted from the column with 20 mM pyridine/acetate buffer and five, 0.5 ml fractions were collected. The fractions were mixed with ScintiVerse and radioactivity was determined by liquid scintillation counting.

Paper chromatography

Descending paper chromatography of radiolabeled enzyme reaction products was performed on Whatman No. 1 filter paper in a solvent system of ethyl acetate:pyridine:acetic acid:water (5:5:1:3). The chromatograms were cut into 1 cm strips, and each strip was mixed with water (0.25 ml) and ScintiVerse (2.5 ml) for determining radioactivity by liquid scintillation counting.

Glycosidase treatments

Enzyme reaction products purified by descending paper chromatography, were treated with glycosidases in 30 μl of 50 mM KH_2PO_4, pH 6.0, containing 0.1 M NaCl and 0.02% NaN_3 for 24 h at 37 °C. Neutral reaction products were treated with 10 μU of *Streptomyces* α1,3/4 fucosidase. Control treatments contained no fucosidase. Sialylated products were subjected to treatment with 10 μU of *Streptomyces* α1,3/4 fucosidase and 20 mU NDV-neuraminidase. In the controls, incubated samples were either untreated or treated with α1,3/4 fucosidase alone. After treatment, the samples were analyzed by descending paper chromatography in a solvent system of ethyl acetate:pyridine:acetic acid:water (5:5:1:3) as described above.

ELISA

ELISAs were performed using buffers and procedures described previously [14, 15]. Microtiter plates were coated with increasing amounts of total detergent extracts of *H. contortus*, *S. mansoni*, COS7, COS7/FTIII, COS7/FTIV cells or human bile-activated lipase (BAL) as antigenic targets. The wells were blocked with a solution of 5% BSA and the presence of Le^x, sLe^x, Le^a, Le^y, Le^b and H-type-1 were probed by incubation with 10 μg ml^{-1} solution of mAb to Le^x and sLe^x or 1:100 dilution of mAb to Le^a, Le^y, Le^b and H-type-1. Bound antibodies were detected by incubating the wells with 1:500 dilution of goat anti-mouse IgM-alkaline phosphatase conjugate for assays employing mAbs to Le^x, sLe^x, Le^y and Le^b and 1:500 dilution of goat anti-mouse IgG-alkaline phosphatase conjugate for assays using mAb to Le^a. Reactive wells were detected by incubation with *p*-nitrophenyl phosphate substrate and measurement of absorbance of each well at 405 nm on a microtiter plate reader (Molecular Devices, Sunnydale, CA). All ELISAs were performed in triplicate and the results represent averages of the three determinations.

Western blot

Total detergent extracts of *H. contortus*, *S. mansoni*, COS7 and COS7/FTIV cells (25 μg) were fractionated by SDS-PAGE on a 10% gel under reducing conditions and transferred to nitrocellulose membranes. The membranes were blocked by incubation in 5% non-fat dry milk solution in TTBS-1 (Tween-20/Tris buffered saline: 10 mM Tris, 300 mM NaCl, 0.05% Tween-20, pH 7.5), then washed four times with TTBS-1 and incubated with 10 μg ml^{-1} solution of mAb to Le^x in dilution solution (TTBS-1 containing 0.5% non-fat dry milk). The treated membranes were washed four times with TTBS-1 and incubated with a 1:10,000 dilution of goat anti-mouse IgM-peroxidase in dilution solution, followed by four washes with TTBS-1. Reactive bands were detected by incubation in ECL chemiluminescent substrate (Amersham Life Sciences, Chicago, IL) and exposure to Biomax MR x-ray film (Eastman Kodak, Rochester, NY) following instructions provided by the manufacturers.

Lectin blots

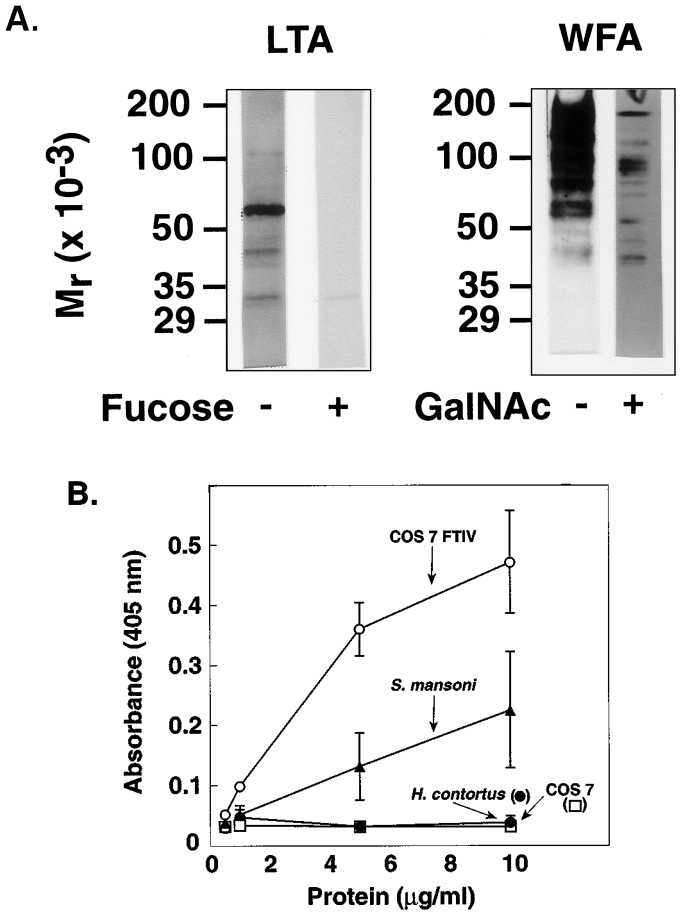
Approximately 3 μg of total detergent extracts of parasites or cells were preabsorbed on streptavidin-Sepharose to remove endogenous biotin-containing proteins, and were then fractionated by SDS-PAGE and transferred to nitrocellulose membranes as described above. The membranes were blocked by incubation with 3% BSA solution in TBS (10 mM Tris, 150 mM NaCl, pH 7.5.), washed four times in TTBS-2 (10 mM Tris, 0.5 M NaCl, 0.3% Tween-20) and incubated in 1 μg ml^{-1} solution of biotinylated *Wisteria floribunda* agglutinin (WFA) or *Lotus tetragonolobus* agglutinin (LTA) in dilution solution of 10 mM Tris, 150 mM NaCl, 0.05% Tween-20 and 1% BSA, with or without 200 mM GalNAc for the WFA and 500 mM fucose for LTA treatments, respectively. The membranes were washed four times with TTBS-2 and incubated with 1:5,000 dilution of streptavidin-peroxidase in dilution solution. The membranes were washed four times with TTBS-2 and reactive bands were visualized by incubation in ECL chemiluminescent substrate and autoradiography as described above. All incubations for both the Western and lectin blots were carried out at room temperature for 1 h and each wash lasted 10 min.

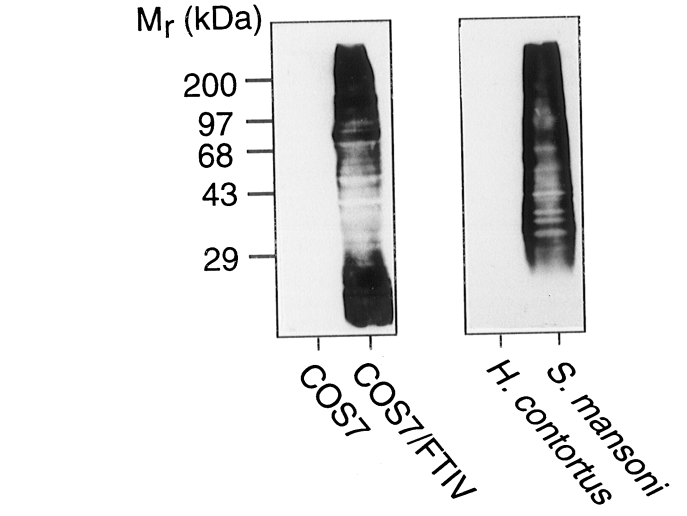
Results

Reactivity of *H. contortus* glycoproteins with specific plant lectins and anti-carbohydrate monoclonal antibodies

We previously reported that *Schistosoma mansoni* and *Dirofilaria immitis* synthesize N-linked oligosaccharides containing outer branch GalNAcβ1 → 4GlcNAc with some of the GlcNAc residues substituted α1,3 with fucose residues [9, 18]. However, *S. mansoni*, but not *D. immitis*, also synthesize glycoproteins containing the Lewis x (Le^x) antigen Galβ1 → 4[Fucα1 → 3] GlcNAcβ1 → R. To determine whether these types of structures are also present in glycoproteins of *Haemonchus contortus*, we used a panel of specific monoclonal antibodies and plant lectins. As shown in Figure 2A, glycoproteins from *H. contortus* were specifically reactive in lectin blots with both WFA and LTA. Although the profiles of glycoproteins recognized show substantial differences, there are some overlaps in size. WFA binds to

glycans containing terminal *β*-GalNAc residues in the LDN motif GalNAc*β*1 → 4GlcNAc [9], while LTA binds to structures containing Fuc*α*1 → 3GlcNAc [26]. Interestingly, we found no detectable reactivity of *H. contortus* extracts with *Ricinus communis* agglutinin (RCA) (data not shown). RCA binds glycans containing terminal *β*1,4 linked Gal residues [27]. In addition, glycoproteins of *H. contortus* did not react with monoclonal antibody to the Le^x antigen, in ELISA (Figure 2B) or Western blot analyses (Figure 2C), whereas *S. mansoni* glycoproteins did react. Additionally, sLe^x and other fucose-containing antigens, Le^a, Le^b, Le^y, H-type 1, could not be detected in extracts of *H. contortus* by ELISA, using monoclonal antibodies specific for each antigen (data not shown). These results indicate that *H. contortus* glycoproteins contain Fuc*α*1 → 3GlcNAc and LDN linkages similar to *D. immitis* and *S. mansoni*. However, *H. contortus* glycoproteins appear to lack *β*1,4 linked Gal residues, and as a consequence, they cannot synthesize the Le^x antigen or other related, fucose-containing Lewis antigens.





Glycosyltransferase activities in extracts of *H. contortus* adults

The above results suggested that *H. contortus* might contain a *β*1,4-*N*-acetylgalactosaminyltransferase (*β*1,4GalNAcT) and an *α*1,3-fucosyltransferase (*α*1,3FT), but not *β*1,4-galactosyltransferase (*β*1,4GalT) activities. Extracts of *H. contortus* adults were assayed for these enzyme activities by incubating extracts of the parasite with the appropriate combination of acceptors and radiolabeled sugar-nucleotide donors as described in "Materials and methods". Extracts of adult *H. contortus* contain both a *β*1,4GalNAcT active toward free GlcNAc and an *α*1,3FT active toward LNnT, but completely lack *β*1,4GalT activity toward free GlcNAc (Figure 3A). This is interesting, because *S. mansoni* was previously shown to contain a *β*1,4GalT activity toward free GlcNAc [28].

In humans and schistosomes Gal*β*1 → 4GlcNAc-containing glycans are typical endogenous acceptors for *α*1,3FT. Because these galactose-containing glycans are lacking in *H. contortus*, we examined the kinetic properties and substrate specificities of the *α*1,3FT in greater detail. The

Figure 2. Reactivity of *H. contortus* extracts with lectins and monoclonal antibodies. (A) Extracts of *H. contortus* were preabsorbed on streptavidin-Sepharose to remove endogenous biotin-containing proteins, separated by SDS/PAGE, and then blotted onto nitrocellulose membrane. Glycosylation was analyzed by lectin-blotting with biotinylated-LTA (left) and biotinylated-WFA (right). Specific binding was determined by including either Fuc to inhibit LTA or GalNAc to inhibit WFA in the blotting mixture, as described in "Materials and methods". (B) Increasing amounts of the indicated cell extracts were absorbed onto microtiter wells and reactivity with anti-CD15 directed to the Le^x antigen was determined by ELISA. Control experiments were performed with extracts of parental COS7 cells, COS7/FTIV cells expressing the human FTIV enzyme and extracts of *Schistosoma mansoni*, which are known to express the Le^x antigen. (C) Indicated cell extracts were subjected to SDS-PAGE, blotted onto nitrocellulose membrane, and expression of the Le^x antigen was monitored by reactivity with anti-CD15 antibodies in Western blot analysis.

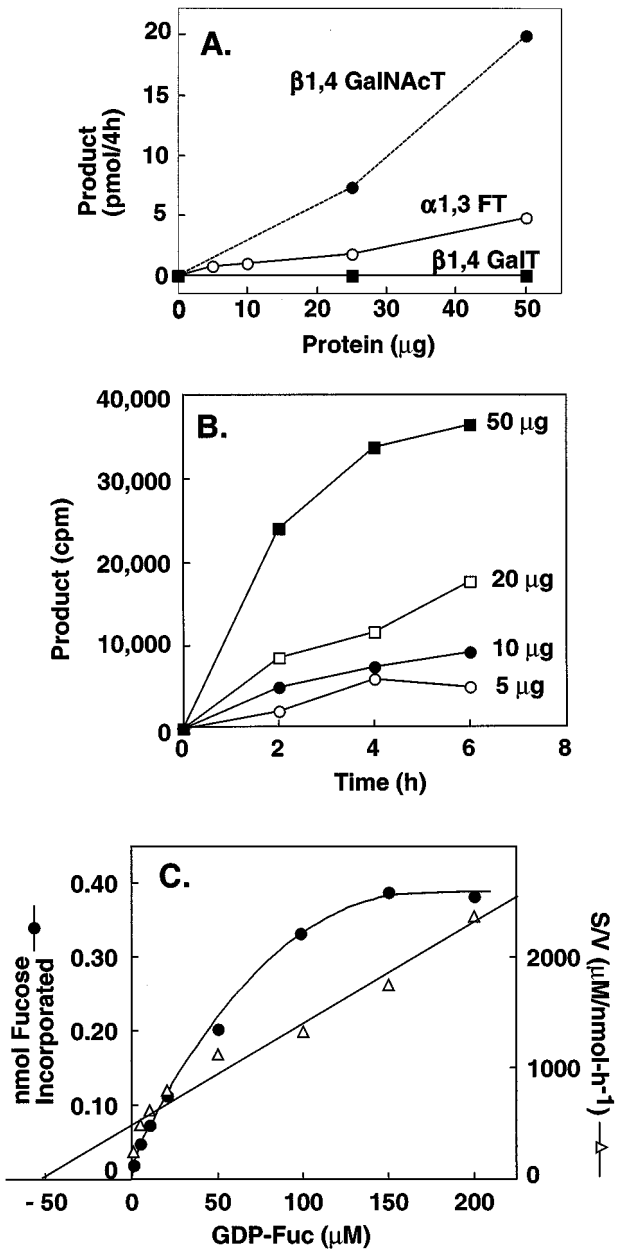


Figure 3. Glycosyltransferase activities in extracts of *H. contortus*. (A) Glycosyltransferase assays were carried out as described in "Materials and methods". β1,4GalT and β1,4GalNAcT assays used UDP-[^{3}H]Gal and UDP-[^{3}H]GalNAc as donors, respectively, and 20 mM GlcNAc as the acceptor. α1,3FT assays used GDP-[^{3}H]-Fuc as the donor and 1.5 mM LNnT as an acceptor. (B) α1,3FT activity with respect to time and protein. (C) *H. contortus* extracts (10 μg) were incubated with increasing amounts of GDP-[^{3}H]-Fuc (50 000 cpm nmol^{-1}) and the acceptor LNnT and the amount of product determined. A Woolf plot of the data is also shown.

α1,3FT in extracts of adult *H. contortus* worms is capable of synthesizing a product from LNnT acceptor and GDP-Fuc acceptor in a manner that is proportional to time of incubation and the amount of extract protein in the assay, except for the highest protein concentration of 50 μg (Figure 3B). The reaction is dependent on the concentration of GDP-Fuc and a Woolf plot of the data [29] demonstrates that the K_m of the enzyme for GDP-Fuc is $\sim$50 μM and the V_{max} is 10.8 nmol mg^{-1} h^{-1} (Figure 3C).

To confirm that the product of the reaction was LNFPIII when LNnT was the acceptor, the product was treated with *Streptomyces* α1,3/4 fucosidase. This enzyme specifically releases fucose residues linked either α1,3 or α1,4 to GlcNAc [30, 31]. The treated and untreated samples were analyzed by descending paper chromatography. As a control, identical treatments were performed with standard ^{3}H-LNFPIII. Untreated samples comigrated with LNFPIII upon paper chromatography. Treatment with *Streptomyces* α1,3/4 fucosidase caused the release of all ^{3}H-fucose from the product (data not shown). These results indicate that crude extracts of *H. contortus* contain an α1,3FT activity capable of synthesizing the Le^x-containing pentasaccharide, LNFPIII, from the tetrasaccharide acceptor, LNnT. In addition, these results show that the only major product is LNFPIII, which makes it unlikely that extracts of the adult organisms contain significant levels of other fucosyltransferases, such as an α1,2-fucosyltransferase that may generate the H-blood group antigen.

Acceptor specificity of *H. contortus* α1,3FT

α1,3FTs in humans exhibit a broad and unique range of acceptor specificities. To evaluate the acceptor specificity of the *H. contortus* α1,3FT, enzymatic reactions were carried out utilizing the acceptors LN, 2,3sLN, 2,6sLN, LNT, and LDN (Figure 1). The *H. contortus* α1,3FT efficiently utilizes the type 2 acceptors, LNnT and LN, to generate LNFPIII and Le^x, respectively, while the type 1 acceptor, LNT, is used poorly (Figure 4). In addition, the enzyme efficiently utilized the disaccharide LDN to generate LDNF. A significant amount of activity is exhibited toward the sialylated acceptor 2,3sLN to generate sLe^x, whereas no significant product is shown from 2,6sLN (Figure 4). These results indicate that the specificity of the *H. contortus* α1,3FT resembles that of human α1,3FT-IV and the α1,3FT in adult schistosomes, except that the *H. contortus* enzyme appears to utilize the 2,3sLN acceptor more efficiently [19–21].

To confirm that the products of the above assays contain the Fucα1 → 3GlcNAc linkages, each product was isolated and treated with *Streptomyces* α1,3/4 fucosidase. Standard trisaccharides Le^x and LDNF were analyzed as controls. The products generated from the acceptors LN and LDN comigrated with Le^x and LDNF, respectively, on paper chromatography. Treatment with α1,3/4 fucosidase caused complete release of ^{3}H-fucose in both products (data not shown). A similar release of ^{3}H-fucose was observed with standard ^{3}H-Le^x and ^{3}H-LDNF (data not shown). These results confirm that the products obtained from the reaction of LN and LDN with the *H. contortus* α1,3FT were the trisaccharides Le^x and LDNF, respectively.

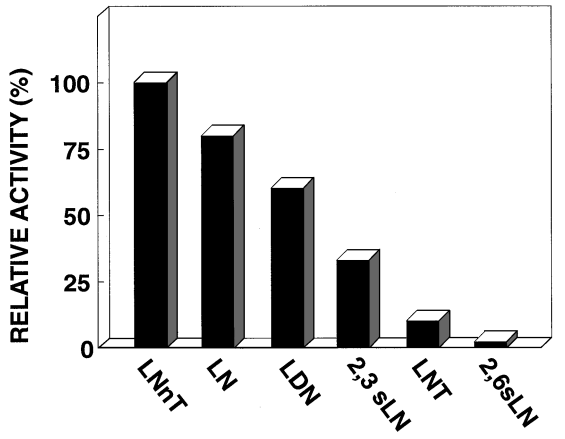


Figure 4. Acceptor specificity of the *H. contortus* α1,3FT. Enzymatic reactions were conducted in which 10 μg *H. contortus* extracts were incubated with 1.5 mM of the indicated acceptors and GDP-[^{3}H]-Fuc. The products formed were quantified as described in "Materials and methods". Values represent the amount of activity relative to that obtained with LNnT as an acceptor.

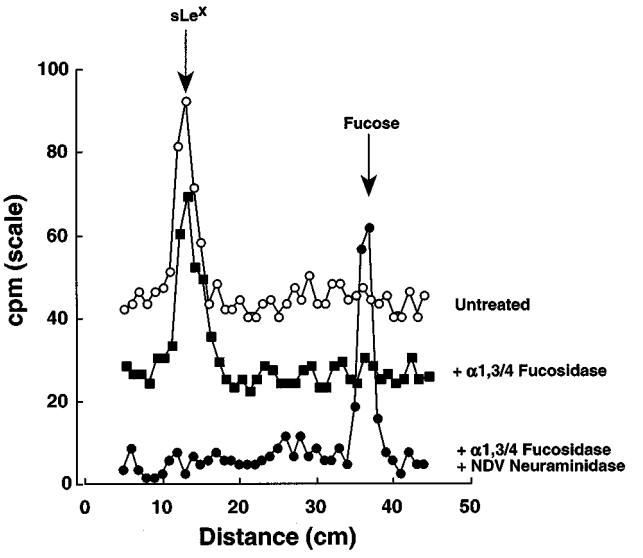


Figure 5. Exoglycosidase treatments of product generated from the acceptor 2,3sLN 2,3sLN was incubated with *H. contortus* extracts and GDP-[^{3}H]-Fuc. The isolated enzyme product, expected to be sLe^x, was either untreated (○); treated with *Streptomyces* α1,3/4 fucosidase alone (■); or treated with both *Streptomyces* α1,3/4 fucosidase and NDV-neuraminidase (●). The samples were then analyzed by descending paper chromatography as described in "Materials and methods".

Similar experiments were performed on the product presumed to be sLe^x obtained with 2,3sLN as the acceptor. The presence of a sialic acid residue blocks release of fucose by the α1,3/4 fucosidase [30, 31]. As expected, the sialylated product was resistant to the α1,3/4 fucosidase and co-migrated on paper with the standard sLe^x (Figure 5). As expected, treatment with neuraminidase alone did not cause release of free ^{3}H-fucose (data not shown). However, when the product was treated sequentially with NDV-neuraminidase, which is specific for α2,3-linked sialic acid, and the *Streptomyces* α1,3/4 fucosidase, ^{3}H-fucose was quantitatively released (Figure 5). These results demonstrate that the product obtained from reaction of the *H. contortus* α1,3FT with 2,3sLN is sLe^x.

NEM sensitivity of the *H. contortus* α1,3*FT*

NEM modifies free sulfhydryl groups on proteins and differentially inhibits the human α1,3/4FTs [22, 32]. To further characterize the *H. contortus* α1,3FT, we performed assays in the presence of increasing concentrations of NEM, utilizing LNnT as the acceptor. Parallel experiments were conducted with COS7/FTIV and COS7/FTIII extracts. The *H. contortus* enzyme was highly resistant to NEM throughout the range of concentrations tested (Figure 6). Similarly, the COS7/FTIV activity was also relatively resistant to NEM, although at higher concentrations, some inhibition was observed. In contrast, the COS7/FTIII activity was inhibited by NEM at low concentrations. These results indicate that the *H. contortus* α1,3FT activity is less sensitive to the effects of NEM than human FTIII and has resistance to NEM resembling that of human FTIV.

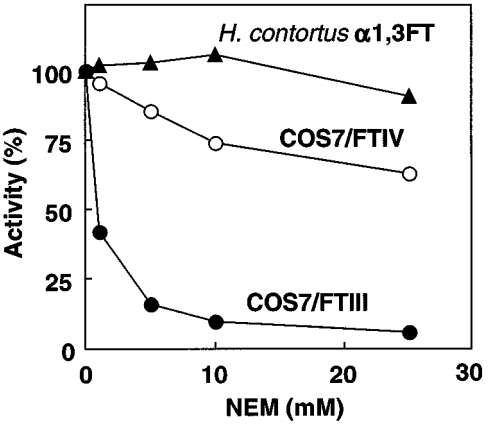


Figure 6. Effects of NEM on the activity of *H. contortus* α1,3FT. Enzyme reactions were conducted in the presence of increasing amounts of NEM and LNnT as the acceptor using either extracts from *H. contortus*, COS7/FTIV or COS7/FTIII cells. The product was determined as described in "Materials and methods".

A single α1,3FT in *H. contortus* extracts appears to fucosylate both LDN and LNnT

Due to limited amounts of *H. contortus* extracts, it was not feasible to purify the α1,3FT to examine whether there are multiple enzymes. However, acceptor competition experiments were performed to address the possibility that different α1,3FTs in *H. contortus* extracts might fucosylate LDN

Table 1. Substrate competition between the acceptors LDN and LNnT for the *H. contortus* α1,3FT

Acceptor	*cpm of Product*[a]	
	LNFPIII	*LDNF*
LNnT (1.5 mM)	6,140	18
LDN (1.5 mM)	20	4,274
LNnT (1.5 mM) + LDN (1.5 mM)	5,409	2,344
LNnT (15 mM) + LDN (1.5 mM)	13,248	424

[a] Each assay contained either LNnT or LDN or a combination, as indicated, along with *H. contortus* extract and GDP-[^{3}H]Fuc (50 000 cpm nmol^{-1}), as described in "Materials and methods". The separate products LNFPIII, generated from LnNT, or LDNF generated from LDN, were isolated and quantified by chromatography on QAE-Sephadex followed by descending paper chromatography. Background radioactivity, defined as cpm obtained in the absence of acceptor (~200 cpm), was subtracted in each determination.

versus LNnT acceptors. If LDN and LNnT are fucosylated by different enzymes, it would be expected that one acceptor could not compete with the other, whereas if they were fucosylated by a single enzyme or class of enzymes, the acceptors should compete with each other. The α1,3FT assays were carried out under conditions in which equimolar amounts of LDN and LNnT (1.5 mM each) were incubated with *H. contortus* extract and GDP-^{3}H-Fuc and products from both acceptors were isolated and quantified. Parallel reaction containing a ten fold molar excess of LNnT to LDN was also performed. Approximately equal amounts of both LDNF and LNFPIII products were also formed with either acceptor alone (Table 1). When both acceptors were present in equimolar concentrations, both LDNF and LNFPIII were generated at roughly equal amounts, although some cross-competition was observed (Table 1). However, when the reaction was carried out with LNnT at molar concentrations ten fold higher than LDN, the amount of LDNF formed was reduced by more than 80%, whereas LNFPIII synthesis was elevated (Table 1). These results suggest that *H. contortus* contains a single α1,3FT or class of enzymes capable of recognizing both LDN and LNnT.

Discussion

Our results demonstrate that *H. contortus* adults contain high levels of an α1,3FT capable of fucosylating either Galβ1 → 4GlcNAc-R, NeuAcα2 → 3Galβ1 → 4GlcNAc-R or GalNAcβ1 → 4GlcNAc to generate Le^x, sLe^x or LDNF, respectively. The *H. contortus* α1,3FT shares similar kinetic properties with the human myeloid enzyme, FTIV and the α1,3FT expressed by adult schistosomes. Interestingly, despite the presence of an α1,3FT activity, no Le^x or sLe^x determinants are present in *H. contortus* glycoconjugates. Synthesis of these specific antigenic determinants would require a β1,4GalT activity to synthesize the appropriate LN-based (Galβ1 → 4GlcNAc-R) acceptors. Our results demonstrate that *H. contortus* adults lack this enzyme activity. However, these parasites do contain both an α1,3FT and β1,4GalNAcT activities capable of synthesizing the LDN motif. In addition, *H. contortus* glycoproteins bind to both LTA and WFA, which recognize Fuc in Fucα1 → 3GlcNAc linkages and GalNAc in GalNAcβ1 → 4GlcNAc linkages, respectively. These combined results make it likely that these parasites generate glycans containing the LDN, rather the LN motif, and can also make fucosylated derivatives of LDN.

We and others have found that *S. mansoni* express glycoconjugates containing abundant amounts of the Le^x, but not sLe^x antigen, and also express fucosylated LDN-type structures [8, 9, 11, 13, 14, 17] We recently characterized an α1,3FT in schistosomes responsible for synthesizing these α1,3-fucosylated determinants and found that the enzyme has many kinetic properties in common with the human FT-IV [19]. In addition, we have found that Le^x is expressed in *S. japonicum* and *S. haematobium* [33]. In contrast, we have found that the Le^x antigen is not expressed in the related trematode *Fasciola hepatica*, the free-living nematode *Caenorhabditis elegans*, or microfilaria and adults of the dog heartworm *Dirofilaria immitis* [33]. The lack of Le^x expression by these helminths is likely to be due not to the absence of a α1,3FT but rather to the lack of β1,4GalT and the consequent inability to synthesize N-acetyllactosamine-containing glycans. Thus, it is likely that these helminths utilize an alternative pathway involving the LDN-motif.

In relation to the possible role of fucose residues as antigenic determinants in *H. contortus*, Haslam *et al.* [6] recently defined the structures of some fucosylated N-glycans in the parasite glycoproteins, including H11, a major integral membrane protein in the microvillar plasma membrane [34, 35]. These glycoproteins contain an unusual Fucα1 → 3GlcNAc linkage in the chitobiosyl core attached to Asn, with one fucose residue on the proximal GlcNAc residue and a second residue on the distal GlcNAc residue, in addition to a Fucα1 → 6GlcNAc linkage on the proximal GlcNAc residue that is commonly found in animal glycoproteins. Such unusual α1,3-fucosylated chitobiosyl core structures have been observed in plant and insect glycoproteins, where they constitute antigenic determinants [36, 37]. It is not yet known whether there are specific α1,3FTs for adding each α1,3 linked fucose to such core-fucosylated N-glycans in *H. contortus* and whether those enzymes are different from the α1,3FT we have identified that acts on terminal LN- and LDN-type structures.

The *H. contortus* α1,3FT exhibits activity similar in many ways to that of human FTIV and the *S. mansoni* α1,3FT [19]. These common features include the more efficient transfer of Fuc to nonsialylated acceptors than to sialylated acceptors and the inability to utilize type-1 acceptors

containing terminal Galβ1 → 3GlcNAc. However, the *H. contortus* α1,3FT can utilize sialylated acceptors more efficiently than either the schistosome α1,3FT or human FTIV [19]. The ability to utilize sialylated acceptors has also been observed with the mouse and chicken homologs of FTIV [38, 39]. Another feature in common with human FTIV is the insensitivity of the *H. contortus* enzyme to inactivation by NEM. The basis of NEM-sensitivity has recently been attributed to the presence of a specific Cys residue in FTIII, FTV, and FTVI, which have been implicated in the binding of GDP-Fuc [40]. This Cys residue is absent in both FTIV and FTVII; however, site-directed mutagenesis of the corresponding amino acid residue to Cys renders FTIV NEM-sensitive. These results suggest that the absence of a NEM-sensitive residue near the GDP-Fuc binding site could be responsible for the NEM resistance of the *H. contortus* enzyme. Identification of the cDNA encoding the *H. contortus* α1,3FT will provide important additional information about its relationship to other known fucosyltransferases.

Coupled with our studies of the α1,3FT activity expressed in adult schistosomes, the results presented in this report suggest that the α1,3FT is a common enzyme of helminthic parasites. α1,3FT activities have been identified in other parasites, lower eukaryotes, and bacteria [41, 42]. Furthermore, it seems these α1,3FTs resemble human FTIV in their kinetic properties, so it is possible that these enzymes are evolutionarily linked to human FTIV. The functions of α1,3-fucosylated glycans with respect to parasite life and survival are unknown at this time. However, the observations that such structures and enzyme activities are highly conserved among many different parasitic species suggests that these fucosylated glycans may be important in worm development and/or host-parasite interactions.

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