**Haemonchus contortus** Glycoproteins Contain N-Linked Oligosaccharides with Novel Highly Fucosylated Core Structures*  

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Structural studies on the N-linked oligosaccharides of *Haemonchus contortus*, an economically important nematode that parasitizes domestic ruminants, have revealed core fucosylation of a type not previously observed in any eukaryotic glycoprotein. Mass spectrometric analyses were performed on detergent extracts of homogenized adult *H. contortus* and on purified H11, a glycoprotein isolated from intestinal brush borders which has been previously shown to be an effective vaccine antigen. The major N-linked glycans identified in the present study have up to three fucose residues attached to their chitobiose cores. The fucoses are found at the 3- and/or 6-positions of the proximal GlcNAc and at the 3-position of the distal GlcNAc. The latter substitution is unique in N-glycans. Most anti-H11 monoclonal antibodies are known to recognize carbohydrate epitopes, and it is possible that the newly discovered multifucosylated core structures are highly immunogenic in this glycoprotein.

*Haemonchus contortus* is an economically important nematode that parasitizes domestic ruminants. Control with chemicals (anthelmintics) is failing due to development of worms resistant to all available products. Nonchemical methods of control are required among which the most attractive is the production of vaccines. Until now, attention has been focused on the identification of immunogenic proteins, but the possibility that parasite carbohydrate antigens could act as novel vaccine candidates has also been hypothesized (1). Indeed there is a considerable body of evidence from studies of a variety of parasitic helminths, e.g. *Schistosoma mansoni* (2) and *Toxocara canis* (3), that glycoconjugasates are important in host-parasite interactions. Indirect evidence from studies of lectin binding, sensitivity of antibody epitopes to periodate oxidation, and susceptibility to peptide-N-glycosidase F (PNGase F) (4–10) has indicated that immunogenic proteins in *H. contortus* are glycosylated but little is known about the nature of the glycans.

It has been proposed that molecular vaccines against *H. contortus* could be based on stimulating the formation of circulating antibodies to identified proteins at the luminal surface of the intestine (11). Of these, a glycoprotein, designated H11, is the most effective vaccine antigen that has been described for any parasitic nematode (12, 13). H11 is the major integral membrane protein in the microvillar plasma membrane (9, 12). It is a type II protein; there are 4 consensus N-glycosylation sites on the extracellular region which accounts for the bulk of its 972 amino acids.2 Injection of sheep with H11 has given over 90% protection in several breeds of sheep including lambs less than 3 months old when challenged, and against challenge with anthelmintic-resistant worms (9, 12, 13, 15). No reports on the glycosylation status of H11 have been published, but it is known that about one quarter of the antibodies produced in response to injection of H11 are anti-carbohydrate.

Rigorous structural studies of *H. contortus* glycoproteins and glycolipids are an essential prerequisite to exploring their possible involvement in protective immunity and their promise as vaccine candidates. To this end we have undertaken a systematic structural analysis of the oligosaccharides of *H. contortus*. In the present work we report structural studies based on fast atom bombardment mass spectrometry which have provided information on N-glycosylation in *H. contortus* glycoproteins. Importantly these studies have uncovered highly unusual core modifications not previously observed in N-linked glycoproteins.

The first step of N-linked biosynthesis in all eukaryotic cells involves the transfer of a dolichol-linked oligosaccharide whose structure is preserved throughout the animal and plant kingdoms. The core domain of this oligosaccharide, which has the structure Manα1,6[Manα1,3]Manβ1,4GlcNAcβ1,4GlcNAc, is retained in the mature glycoprotein, while the peripheral region is extensively remodeled to give an enormous variety of glycans. The core itself is acted upon by a limited set of glycosyltransferases which differ between the plant and animal kingdoms. In mammalian glycoproteins the core can be substituted with a bisecting GlcNAc attached to O-4 of the β-Man and/or Fuc linked to O-6 of the reducing terminal GlcNAc (16, 17). Plant glycoproteins appear to lack the bisecting GlcNAc and fucosylation occurs at O-3 rather than O-6 of the terminal GlcNAc (18). In addition, plant structures are frequently substituted with Xyl attached at O-2 of the β-Man. Relatively few invertebrate glycoproteins have been rigorously characterized.
and the full diversity of their core modifications remains to be established. Available information suggests that invertebrates have features in common with both mammalian and plant glycoproteins. Importantly they are capable of synthesizing both α1,3 and α1,6 fucosyl linkages on the core, and these two linkages can be present in the same glycan (Fig. 1) (19).

In this study we report the discovery of a new type of core fucosylation in *H. contortus* glycoproteins in which Fuc is attached to the distal GlcNAc of the chitobiose moiety, resulting in glycans which are substituted with up to three Fuc residues on the core.

**EXPERIMENTAL PROCEDURES**

**Production of *H. contortus***—Worm-free lambs were infected with a UK anthelmintic-susceptible isolate of *H. contortus*. Feces were collected from bagged lambs and cultured at room temperature for 14 days. Infective (L3) larvae were collected by Baermann apparatus, purified by sucrose gradient centrifugation, and thoroughly washed with tap water. After humanely killing animals, adult worms were collected by Baermann apparatus at 38 °C in Earle’s balanced salt solution and hand picked free of any remaining abomasal debris. Worms were stored at −80 °C prior to analysis.

**Detergent Extraction of *H. contortus***—Approximately 5 g of adult *H. contortus* were homogenized on ice in an extraction buffer of 0.5% w/v cetyltrimethylammonium bromide, in 0.1 M Tris (pH 7.4), and extracted for a further 24 h at 4 °C. Solid debris were removed by centrifugation at 3000 rpm for 10 min. Detergent was removed by extensive dialysis against 50 mM ammonium bicarbonate buffer (pH 7.6).

**Preparation of H11**—H11-enriched extracts in the detergent Thesit (Boehringer Mannheim) were prepared as described elsewhere (9, 12).

**Reduction and Carboxymethylation**—Reduction and protection of the disulfide bridges of the detergent extracted proteins of *H. contortus* was carried out as described previously (20).

**Tryptic Digest**—The reduced carboxymethylated *H. contortus* proteins were digested with t-1-tosylamide-2-phenylethylchloromethyl ketone bovine pancreas trypsin (EC 3.4.21.4, Sigma), for 5 h at 37 °C in 50 mM ammonium bicarbonate buffer (pH 8.4). The products were purified by Sep-Pak C18 (Waters Ltd.) as described elsewhere (20).

**Preparation of CNBr Fragments of H11**—Detergent was removed from purified H11 by extensive dialysis against 50 mM ammonium bicarbonate buffer (pH 7.6) and then lyophilized. The lyophilized sample (approximately 250 μg), was dissolved in 100 μl of a solution of CNBr in 70% formic acid and left in the dark for 5 h. The reaction was terminated by drying in vacuo. after the addition of 500 μl of water. The resulting peptide mixture was purified on a Sep-Pak C18 (Waters Ltd.) as described previously (20).

**PNGase F Digestion**—PNGase F (EC 3.5.1.52, Boehringer Mannheim) digestion was carried out in ammonium bicarbonate buffer (50 mM, pH 8.4) for 16 h at 37 °C using 0.6 unit of the enzyme. The reaction was terminated by lyophilization and the products were purified on Sep-Pak C18 (Waters Ltd.) as described elsewhere (20).

**PNGase A Digestion**—Glycopeptides remaining after PNGase F digestion were further digested with PNGase A (EC 3.5.1.52, ICN), in ammonium acetate buffer (50 mM, pH 5.0), for 16 h at 37 °C using 0.2 milliunit of the enzyme. The reaction was terminated by lyophilization and the products were purified on a Sep-Pak C18 (Waters Ltd.) as described previously (20).

**Exo-glycosidase Digestions**—These were carried out on released glycans using the following enzymes and conditions: α-mannosidase (from jack bean, EC 3.2.1.24, Boehringer Mannheim), 0.5 unit, and β-mannosidase (from snail, EC 3.2.1.25, Sigma), 0.1 unit in 100 μl of 50 mM ammonium acetate buffer, pH 4.25, for a total of 24 h, a fresh aliquot of enzymes being added after 12 h; α-L-fucosidase (from bovine kidney, EC 3.2.1.51, Boehringer Mannheim), 0.2 unit in 100 μl of 50 mM ammonium acetate buffer, pH 4.5–5.0, for 24 h. All enzyme digestions were incubated at 37 °C and terminated by lyophilization. An appropriate

**SCHEME 1. Summary of overall experimental strategy employed to characterize *H. contortus* N-glycans.**
aliquot was taken after each digestion and permethylated for FAB-MS analysis after purification on a Sep-Pak C18 (Waters Ltd.).

Methanolysis—The reagent was prepared by bubbling dry HCl gas into methanol as described previously (20). After cooling, a 20-μl aliquot of this reagent was added to the permethylated sample for 1 min at room temperature. A 1-μl aliquot was removed for FAB-MS analysis, and the remainder of the sample was dried under nitrogen.

Chemical Derivatization for FAB-MS—Permethylated using the sodium hydroxide procedure was performed as described elsewhere (20).

FAB-MS Analysis—FAB mass spectra were acquired as described previously (20). CAD MS-MS collision activated decomposition spectra were recorded using a Fisons VG Analytical four-sector ZAB-T mass spectrometer in the array detector mode as described elsewhere (21).

RESULTS

Structural studies were performed on detergent extracts of homogenized adult *H. contortus* and on purified H11 glycoprotein isolated from intestinal brush borders. Scheme 1 summarizes the overall strategy employed.

FAB-MS of N-Glycans Released from Detergent Extracts by PNGase F and PNGase A—Detergent extracts were digested sequentially with trypsin and PNGase F. The latter enzyme is capable of releasing all known N-linked oligosaccharides except those with fucose attached to the 3-position of the Asn-linked GlcNAc residue. Such PNGase F-resistant oligosaccharides have been found to be sensitive to PNGase A, an enzyme found in almond emulsin (22, 23). PNGase F-released glycans were separated from peptides and glycopeptides and were analyzed by FAB-MS after permethylation (Fig. 2A and Table I). The peptide/glycopeptide fraction was further digested with PNGase A and released glycans were similarly analyzed (Fig. 2B and Table I). Notable features of these data are: (i) the majority of PNGase F released glycans have compositions consistent with high mannose structures (Hex5–9HexNAc2) or truncated cores with and without fucose (Fuc0–2Hex2–4HexNAc2); (ii) the majority of PNGase A released glycans have compositions consistent with truncated cores carrying up to three fucoses (Fuc1–3Hex2–4HexNAc2); (iii) very minor components are present in both glycan pools whose compositions are consistent with structures bearing short complex-type antennae, e.g. HexNAc-, HexHexNAc-, HexNAc2-, and FucHexNAc2 whose presence is confirmed by A-type fragment ions (Table I). Among these compositions, Fuc2Hex2–3HexNAc2 from the PNGase F digest and Fuc3Hex2–3HexNAc2 from the PNGase A digest were unexpected because none corresponded to known core structures.

Linkage Analysis of Oligosaccharides Released by PNGase F and A—Data from linkage analyses of released glycans are summarized in Table II. These results are fully consistent with high mannose and truncated structures being the major constituents of the N-glycan population. Of particular interest are the data from the variously linked GlcNAc residues (Fig. 3). PNGase F-released glycans gave 4-GlcNAc, 3,4-GlcNAc, and 4,6-GlcNAc while those released by PNGase A contained an
additional 3,4,6-GlcNAc. The high abundance of 3,4-GlcNAc is notable because the FAB data from this sample (Fig. 2A and Table I) suggested that components with complex-type antennae having compositions consistent with a branched GlcNAc, i.e. FucHexHexNAc and FucHexNAc2, are relatively minor. This apparent anomaly was resolved by further characterization of the fucosylated core structures (see below). The presence of 3,4,6-GlcNAc in the PNGase A sample is indicative of the presence of the unusual difucosylated core discovered in honeybee phospholipase A2 (Fig. 1).

Characterization of Fucosylated Core Structures—In order to facilitate structural characterization of truncated components with unusual Fuc compositions, the glycan pools were subjected to digestion with α- and β-mannosidase, and the products were examined by FAB-MS after Sep-Pak purification (Fig. 4 and Table III). The FAB data indicated that, as expected, the high mannose and nonfucosylated truncated structures were fully trimmed to chitobiose (m/z 559), while fucosylated truncated structures were variably trimmed and putative complex-type structures were unaffected (Table III). Other than chitobiose, the major core structures present in the PNGase F pool after the mannosidase digestions were Fuc1HexNAc2, Fuc1HexHexNAc2, Fuc1HexHexNAc2, Fuc1HexHex2HexNAc2, and Fuc2HexHexNAc2. Of these, the difucosylated components are the most interesting because they are unlikely to have the two fucoses on the reducing GlcNAc (see Fig. 1) because of the resistance of this compound to PNGase F digestion (22).

The spectra of the mannosidase-digested PNGase A sample were characterized by major molecular ions at m/z 907 and 1285, consistent with compositions FucHexNAc2 and FucHexHexNAc2, respectively. The former is the product expected from trimming of difucosylated glycans having the two fucoses attached to the reducing GlcNAc. The composition of the latter indicates that Fuc3Hex1HexNAc2 in the original sample (see Fig. 2B and Table I) are trimmed to Fuc1Hex1HexNAc2 by the mannosidase digestion. Interestingly Fig. 4C contains an abundant A-type fragment ion at m/z 638 (FucHexHexNAc2), together with a secondary fragment ion at m/z 432 (corresponding to elimination of Fuc from m/z 638). Taking into consideration the relative abundances of the various molecular ions we considered it probable that these fragment ions were derived from the trifucosylated component. To explore this further, the trifucosylated component was subjected to CAD MS-MS experiments (Fig. 5). The [M + H]+ ion gave

### Table I

Assignments of molecular and fragment ions observed in FAB spectra of permethylated N-glycans of *H. contortus*

| Signal m/z | Assignment |
|------------|------------|
| 260 HexNAc+ | Loss of methanol gives m/z 432 |
| 464 HexHexNAc+ | Loss of methanol gives m/z 473 |
| 505 HexNAc+ | Loss of methanol gives m/z 636 |
| 668 HexHexNAc+ | Loss of methanol gives m/z 840 |
| 842 HexHex2HexNAc+ | Loss of methanol gives m/z 1248 |
| 1076 HexHexNAc+ | Loss of methanol gives m/z 1285 |
| 1119 HexHex2HexNAc+ | Loss of methanol gives m/z 2064 |
| 1199 HexHexNAc+ | Loss of methanol gives m/z 2096 |
| 1208 HexHexNAc+ | Loss of methanol gives m/z 2097 |
| 1293 Fuc1HexHexNAc2+H+ | Loss of methanol gives m/z 2417 |
| 1323 Fuc1HexHexNAc2+H+ | Loss of methanol gives m/z 2604 |
| 1353 HexHexHexNAc2+H+ | Loss of methanol gives m/z 2872 |
| 1394 HexHexHexNAc2+H+ | Loss of methanol gives m/z 3041 |
| 1467 HexHexHexNAc2+H+ | Loss of methanol gives m/z 3210 |
| 1484 HexHexHexNAc2+H+ | Loss of methanol gives m/z 3379 |
| 1497 Fuc1HexHexNAc2+H+ | Loss of methanol gives m/z 3548 |
| 1527 HexHexHexNAc2+H+ | Loss of methanol gives m/z 3717 |
| 1557 HexHexHexNAc2+H+ | Loss of methanol gives m/z 3886 |
| 1671 HexHexHexNAc2+H+ | Loss of methanol gives m/z 4055 |
| 1701 HexHexHexNAc2+H+ | Loss of methanol gives m/z 4224 |
| 1761 HexHexHexNAc2+H+ | Loss of methanol gives m/z 4393 |
| 1813 Fuc1HexHexNAc2+H+ | Loss of methanol gives m/z 4562 |
| 1892 HexHexHexNAc2+H+ | Loss of methanol gives m/z 4731 |
| 1965 HexHexHexNAc2+H+ | Loss of methanol gives m/z 4900 |
| 1987 Fuc1HexHexNAc2+H+ | Loss of methanol gives m/z 5069 |
| 2086 HexHexHexNAc2+H+ | Loss of methanol gives m/z 5238 |
| 2191 Fuc1HexHexNAc2+H+ | Loss of methanol gives m/z 5407 |
| 2373 HexHexHexNAc2+H+ | Loss of methanol gives m/z 5576 |

### Table II

GC-MS analysis of partially methylated alditol acetates obtained from the PNGase F and A released N-glycans of *H. contortus*

| Elution time (min) | Characteristic fragment ions | Assignment |
|-------------------|-------------------------------|------------|
| 17.07             | 115, 118, 131, 162, 175       | Terminal fucose, major |
| 18.70             | 102, 118, 129, 145, 161, 162, 205 | Terminal mannos, major |
| 19.97             | 102, 118, 129, 145, 161, 162, 205 | Terminal galactose, major |
| 19.92             | 129, 130, 161, 190            | 2-Linked mannos, major |
| 20.22             | 118, 129, 161, 234            | 3-Linked mannos, minor |
| 20.37             | 99, 102, 118, 129, 162, 189, 233 | 6-Linked mannos, minor |
| 21.59             | 129, 130, 189, 190            | 2,6-Linked mannos, minor |
| 21.70             | 118, 129, 189, 234            | 3,4-Linked mannos, major |
| 22.79             | 117, 159, 203, 205            | Terminal GlcNAc, major |
| 23.26             | 117, 159, 203, 205            | Terminal Gal NAc, minor |
| 23.72             | 117, 159, 233                | 4-Linked GlcNAc, major |
| 24.60             | 117, 159, 346                | 3,4-Linked GlcNAc |
| 25.08             | 117, 159, 261                | 4,6-Linked GlcNAc |
| 25.99             | 117, 159                    | 3,4,6-Linked GlcNAc |

* PNGaseA only.
FIG. 4. FAB-mass spectrum of permethylated N-glycans from *H. contortus* after α- and β-mannosidase digestion. *H. contortus* N-glycans were released from glycopeptides by digestion with PNGase F (A and B), or PNGase A after an initial PNGase F digest (C and D). The released glycans were digested with α- and β-mannosidase, permethylated, and purified by Sep-Pak. The 35% (v/v) (A and C) and 50% (v/v) (B and D) acetonitrile fractions were screened by FAB-MS.
important daughter ions at m/z 638 (FucHexHexNAc<sup>+</sup>), 450 (loss of Fuc from m/z 638 by β-cleavage), 432 (elimination of Fuc from m/z 638), 420 (loss of Hex from m/z 638 by β-cleavage), 402 (elimination of Hex from m/z 638) and 156 (Fuc<sup>−</sup> minus methanol) (Fig. 5A). These data are consistent with structure I (Fig. 5, inset) for which we propose novel fucosylation on the distal GlcNAc of the chitobiose core. Corroborative data were afforded by CAD-MS-MS on the [M + Na]<sup>+</sup> molecular ion whose major daughters are derived from ring cleavage reactions (Fig. 5B, inset) (24).

Further evidence for novel core fucosylation came from CAD-MS-MS studies of difucosylated components present in the mannosidase digests (Fig. 6). The most striking feature of these data are the marked differences between the daughters formed from parents of the same mass depending on whether the oligosaccharide was originally released by PNGase F or A. The [M + H]<sup>+</sup> ions at m/z 1089 afforded major A-type ions at m/z 638 (FucHexHexNAc<sup>+</sup>) (Fig. 6A) and m/z 464 (HexHexNAc<sup>+</sup>) (Fig. 6B) for PNGase F-released and PNGase A-released material, respectively, indicative of differing fucose substitution in the two samples. Similar striking differences were observed in the daughter ion spectra of the [M + Na]<sup>+</sup> quasimolecular ions (Fig. 6, C and D). The structurally important daughter ions in these spectra are m/z 671 and 676. The former is the product of concerted elimination of mannose and fucose from the distal GlcNAc (Scheme 2, structures II and IV), while the latter is diagnostic of a difucosylated proximal GlcNAc (Scheme 2, structure III). In summary, the CAD-MS-MS data suggest that difucosylated oligosaccharides released by PNGase F carry a single fucose on each GlcNAc residue of the core while the PNGase A-released material is a mixture in which either a single fucose is attached to each GlcNAc or both fucoses are on the proximal GlcNAc. Further, the absence of a secondary cleavage ion corresponding to loss of methanol from the A-type ion at m/z 1231 is due to loss of methanol from the m/z 1263 molecular ion (A). The fragment ions were magnified by × 50.

**Fig. 5.** Positive ion CAD mass spectra of the *H. contortus* trifucosylated core structure after collision activation of the (M + H)<sup>+</sup> (A) and (M + Na)<sup>+</sup> (B) molecular ions. The fragment ion at m/z 1231 is due to loss of methanol from the m/z 1263 molecular ion (A). The fragment ions were magnified by × 50.
The latter experiment provides useful data pertaining to the original sites of attachment of the fucosyl residues because each “tagged” attachment site is identifiable by a mass shift of the relevant fragment ion in the electron impact mass spectrum. The above mild methanolysis strategy was applied to the mannosidase-digested, methylated samples described earlier (see Fig. 4). As expected the components with more than one fucose were rapidly degraded to monofucosylated oligosaccharides. The PNGase F-derived material gave major \([M+H]^+\) ions at \(m/z\) 937 and 940, corresponding to FucHexHexNAc\(_2\) with zero and one deuteromethyl groups, respectively, while the major signals from PNGase A-derived material were at \(m/z\) 940 and 943, consistent with one and two deuteromethyl groups, respectively (data not shown). Analogous data were obtained for FucHex\(_2\)HexNAc\(_2\), which showed incorporation of one and two deuteromethyl groups in the PNGase F and A samples, respectively (data not shown). These data indicate that the difucosylated glycans in the PNGase F-digested material have a single very labile fucose, while the trifucosylated glycans in the PNGase A-digested material have two very labile fucose residues. The labile fucoses are expected to be 3-linked to GlcNAc, and therefore these results are fully in accordance with the structures deduced from the MS-MS experiments (see above). Further evidence for the novel fucose on the distal GlcNAc being attached at the 3-position was provided by linkage analysis. The spectrum of 4-linked GlcNAc (Fig. 7) showed a deuterium isotope pattern consistent with a deuteromethyl group being attached at the 3-position in a portion of the sample (Fig. 7, inset).

The anomic configurations of the fucose residues were investigated by beef kidney \(\alpha\)-fucosidase digestion of the PNGase F- and A-glycan pools. The products were permethylated and analyzed by FAB-MS. Comparison of the spectra (data not shown) with those in Fig. 2 revealed that mono- and difucosylated components in the PNGase F sample were fully defucosylated, thus establishing that the novel fucose on the distal GlcNAc is \(\alpha\)-linked. Digestion was less complete in the PNGase A sample, which lost the molecular ions for trifucosylated oligosaccharides but retained molecular ions for mono- and difucosylated structures. It is probable that the multifucosylated cores are difficult to digest for steric reasons.

Taken together, the above data are consistent with \textit{structures} V–VIII (Fig. 8) being the core sequences of a major family of N-glycans present in glycoproteins from adult \textit{H. contortus}.

\textbf{Analysis of N-Linked Oligosaccharides from H11 Glycoprotein—}Because H11 was found to be insoluble in the buffers used for tryptic digestion, this material was digested with cyanogen bromide prior to release of the N-glycans (Scheme 1). Subsequent analyses were identical to those carried out on the total detergent extract. The FAB spectra of the PNGase F- and A-released oligosaccharides (Fig. 9) contain major ions corresponding to high mannose and truncated structures. Importantly all the components having masses corresponding to novel compositions in detergent extracted material (Fig. 2 and
Table I) are also present in the H11 sample. The structures of the di- and trifucosylated oligosaccharides from H11 were explored by FAB-MS, linkage analysis, enzyme digests, and mild methanolysis. All structural data were consistent with structures V, VI, VII, and VIII (Fig. 8) being present in H11 (data not shown).

DISCUSSION

The results presented in this report include the discovery of a new highly fucosylated core structure in glycans from detergent extracts of adult worms and from the gut glycoprotein H11. The new structures have been submitted to the Complex Carbohydrate Structure Data base (CCSD) (25). Fucosylation at the 3- and 6-positions of the reducing terminal GlcNAc and at the 3-position of the distal GlcNAc results in structures with up to three fucosyl residues on the chitobiose core. This remarkable degree of core substitution has not previously been observed in any eukaryotic glycoprotein and is expected to confer unique properties. The novel glycans have between one and four mannose residues attached to the fucosylated chitobiose structures. Data from α- and β-mannosidase digestions and linkage studies suggest that these mannoses are linked in the manner expected for eukaryotic glycoproteins, i.e. they have between zero and three α-mannoses attached to the β-mannose of structures V–VIII in Fig. 8.

N-Glycans difucosylated on the reducing terminal GlcNAc have previously been described on honeybee venom phospholipase A2 (19), but an additional fucosylation event on the distal GlcNAc is unique. There is one previous report of unusual substitution of the distal GlcNAc in the core of an N-glycan, but this was discovered in a mutant cell line and has not yet been observed in normal glycoproteins (26). Thus, Stanley and colleagues have shown that Lec 18, a mutant Chinese hamster ovary cell line, expresses altered cell surface N-glycans with a GlcNAc residue substituting the 6-position of the distal core GlcNAc residue. Interestingly the substituted core structure in the Lec 18 cell line and also another mutant cell line Lec 14 (27), which has a GlcNAc substituting the 2-position of the β-mannose, showed increased resistance to N-acetyl-β-D-hexosaminidase. Similar resistance to exoglycosidase digestion of the core was observed in the present study (Fig. 3 and Table III), supporting the hypothesis that the crowded cores are sterically inaccessible to some enzymes.

The presence of up to two 3-linked fucose residues on the core of the H. contortus N-linked glycans is of considerable interest.
The Fucα(1–3)GlcNAc moiety has been shown to be a highly antigenic epitope of both plant (28) and insect (29) glycoproteins and also accounts for the immunogenic cross-reactivity between plant and insect glycoproteins (29). This structure has also been shown to be a major allergenic determinant in phospholipase A2, and individuals who are allergic to honeybee venom produce appreciable levels of IgE antibodies to the 3-linked fucose of the N-glycans of this glycoprotein (19, 30). In addition, Fucα(1–3)GlcNAc is an important component of the Lex, Leα, Sialy-Lex, and VIM-2 blood group determinants, which have been implicated in selectin-mediated trafficking of lymphocytes, inflammatory processes, apoptosis, and other cell-cell interactions in mammals (14, 31, 32).

Prior to the present study, knowledge of *H. contortus* protein glycosylation has been limited to results from immunological probes and lectin binding studies (4–13, 15). Of particular interest was a 143-kDa immunogenic protein found in adult stage worms which bound concanavalin A and *Helix pomatia* agglutinin but was resistant to N- and O-glycosidase digestion (5, 6). Other workers have shown that a 70–90-kDa immunogenic protein found on the surface of *H. contortus* larvae binds wheat germ agglutinin, but is resistant to peptide N-glycosidase F (7). Jasmer et al. (8) raised a series of monoclonal antibodies to gut surface antigens, and two selected monoclonal antibodies were shown to react with carbohydrate antigens by the sensitivity of the epitope to periodate oxidation. Attempts to remove the carbohydrate epitope with peptide N-glycosidase F and O-glycanase failed. It is probable that many of the putative carbohydrate epitopes described in the above studies correspond to the N-glycans reported in this report. In particular, the frequently observed insensitivity to peptide N-glycosidase F is explained by fucosylation at the 3-position of the proximal GlcNAc of the core (22).

The structural studies reported in this study should facilitate research addressing the importance of glycosylation in host-nematode interactions and the possible exploitation of novel glycan epitopes in vaccine development. It is clear from our results on H11 that the transferases involved in biosynthesizing the novel multifucosylated cores are expressed in gut tissue of *H. contortus*. However, whether the novel glycans are also associated with the cuticle surface or other areas of the adult worm remains to be established.

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**FIG. 9.** FAB-mass spectrum of permethylated N-glycans from H11. N-Glycans were released from H11 glycopeptides by digestion with PNGase F (A), or PNGase A after an initial PNGase F digest (B).
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