Structural Studies of N-Glycans of Filarial Parasites

CONSERVATION OF PHOSPHORYLCHOLINE-SUBSTITUTED GLYCANS AMONG SPECIES AND DISCOVERY OF NOVEL CHITO-OLIGOMERS*

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N-Type glycans containing phosphorylcholine (PC-glycans), unusual structures found in the important human pathogen filarial nematodes, represent a novel target for chemotherapy. Previous work in our laboratories produced compositional information on the PC-glycan of ES-62, a secreted protein of the rodent parasite Acanthocheilonema viteae. In particular, we established using fast atom bombardment mass spectrometry (MS) analysis that PC was attached to a glycan with a trimannosyl core, with and without core fucosylation, carrying between one and four additional N-acetylgalactosamine residues.

In the present study, we demonstrate that this structure is conserved among filarial nematodes, including the parasite of humans, Onchocerca volvulus, for which new drugs are most urgently sought. Furthermore, by employing a variety of procedures, including collision-activated dissociation MS-MS analysis and matrix-assisted laser desorption MS analysis, we reveal that surprisingly, filarial nematodes also contain N-linked glycans, the antennae of which are composed of chito-oligomers. To our knowledge, this is the first report of such structures in a eukaryotic glycoprotein.

Adult filarial nematodes secrete phosphorylcholine (PC) containing glycoproteins during parasitism of their vertebrate hosts (1). A number of studies performed in vitro lead to the conclusion that this PC may have an immunomodulatory function. Thus, PC-containing filarial products have been shown to (i) inhibit proliferation of human T-cells induced by phytohemagglutinin (2) and also murine B cells induced via the antigen receptor (3), and (ii) modulate a number of signal transduction elements associated with the antigen receptor, including various isoforms of protein kinase C, several protein tyrosine kinases, phospholipase D, Rac, phosphoinositide 3-kinase and mitogen-activated protein kinase in either or both of murine B cells and the human T-cell line Jurkat (3–6). As these effects appear to be largely due to the PC moiety of the molecules, we are interested in the possibility of developing novel anti-filarial drugs that prevent PC attachment during biosynthesis. Structural and biosynthetic information on PC-containing filarial biopolymers is an essential prerequisite to rational design of such drugs.

ES-62 is the major PC-containing protein secreted by Acanthocheilonema viteae, a rodent filarial nematode (7). We have recently shown that PC is attached to the N-linked glycans in ES-62 (8, 9), almost certainly being added in the Golgi during intracellular trafficking following the generation of an appropriate glycan substrate (10). Mass spectrometric structural analyses have defined the types of N-glycan in ES-62 that carry PC substitution (11). They all have a trimannosyl core, which is characteristic of eukaryotic N-glycans, and a portion are core-fucosylated. In addition they carry between one and four N-acetylgalactosamine (GlcNAc) residues that are separately attached as antenna “stubs” to the core. It is likely that PC is attached directly to one or more of these GlcNAc residues (11).

Although such information on structure/biosynthesis may be employed in the design of inhibitors of PC attachment to ES-62, it is unknown whether PC is attached to similar glycan structures on all filarial nematodes, and hence the relevance to drug development for human filarial parasites is uncertain. To address this issue, we have begun to screen a range of filarial parasites for their N-glycan content. In this study, we compared the major N-glycans present in extracts of adult A. viteae with similar extracts from Onchocerca volvulus, the human filarial nematode for which safe and effective drugs against adult stages is most urgently sought (12), and with secreted material from the closely related (13) bovine parasite Onchocerca gibsoni, which has been employed for drug screening (14). We show that the two Onchocerca species have PC containing glycans of the type identified in A. viteae, indicating that PC-glycans could be a target for the development of wide-spectrum anti-filarial drugs. Furthermore, we report the unexpected discovery of highly unusual N-linked glycans, the antennae of which are composed of chito-oligomers. All three nematodes show remarkable similarity in their chito-oligomer content.

EXPERIMENTAL PROCEDURES

Parasite Material—Adult A. viteae (mixed males and females) were recovered from jirds (Meriones libycus) infected eight weeks previously according to the method of Worms et al. (15). The worms were washed several times in sterile RPMI 1640 medium before being homogenized in phosphate-buffered saline (pH 7.2) containing proteolytic enzyme inhibitors as described previously (16). The extract was centrifuged (20,000 × g for 25 min), and the supernatant was recovered and stored at −20 °C until used. Adult O. volvulus were recovered from nodules (frozen) surgically removed from Guatemalan patients. A phosphate-
buffered saline extract was prepared according to the method employed with *A. viteae*. Adult female *O. gibsoni* were recovered from nodules excised from infected Queensland cattle, washed thoroughly with RPMI 1640 medium, and then incubated for 48 h at 37 °C. The spent medium was recovered, passed through a 0.2 mm membrane, and excretory secretory material was prepared as described previously (17).

**Reduction and Carboxymethylation**—Reduction and protection of the disulfide bridges of the filarial proteins was carried out as described (18, 19).

**Tryptic Digest**—The reduced carboxymethylated filarial proteins were digested with L-1-tosylamide-2-phenylethylchloromethyl ketone-treated bovine pancreas trypsin (EC 3.4.21.4) (Sigma) for 5 h at 37 °C in 50 mM ammonium bicarbonate buffer (pH 8.4).

**Peptide N-Glycosidase F (PNGase F) Digestion**—PNGase F (EC 3.2.2.18) (Roche Molecular Biochemicals) digestion was carried out in ammonium bicarbonate buffer (50 mM, pH 8.4) for 16 h at 37 °C using 0.6 units of the enzyme. The reaction was terminated by lyophilization and the products were purified on Sep-Pak C18 (Waters Corp.) as described (18, 19).

**Hydrogen Fluoride Treatment**—Samples were incubated with 50 µl of 48% HF (Aldrich) at 0 °C for 48 h (11). The reagent was removed under a stream of nitrogen.

**Exoglycosidase Digestion**—Released glycans were digested with *N*-acetyl-β-d-hexosaminidase (from bovine kidney) (EC 3.2.1.30) (Roche Molecular Biochemicals): 0.2 units in 100 µl of 50 mM sodium citrate phosphate buffer at pH 4.6 for 18 h at 37 °C. The reaction was terminated by lyophilization, and the reaction products were permethylated for FAB-MS analysis (18, 19).

**Chemical Derivatization for FAB-MS and GC-MS Analysis**—Permethylated using the sodium hydroxide procedure was performed as described (18, 19). Perdeuteroacetylated derivatives were prepared from permethylated samples for GC-MS linkage analysis as described (20).

**GC-MS Analysis**—GC-MS analysis was carried out on a Fisons Instruments MD800 machine fitted with a DB-5 fused silica capillary column (30 m x 0.32 mm internal diameter, J & W Scientific). The partially methylated alditol acetates were dissolved in hexanes prior to on-column injection at 65 °C. The GC oven was held at 65 °C for 1 min before being increased to 290 °C at a rate of 8 °C/min.

**FAB-MS Analysis**—FAB-MS spectra were acquired using a ZAB-2S.E. 2FPD mass spectrometer fitted with a caesium ion gun operated at 30 kV. Data acquisition and processing were performed using the VG Analytical Opus software. Solvents and matrices were as described (18, 19, 21). 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 (19).

**MALDI-MS Analysis**—MALDI-MS was performed in the Reflectron mode using a Perseptive Biosystems Voyager Elite mass spectrometer with delayed extraction. Samples were dissolved in 20 µl of 80:20 (v/v) methanol/water, and aliquots (0.5 µl) of the resulting solutions were analyzed using a matrix of 2,5-dihydrobenzoic acid. Angiotensin and insulin B chain were employed as external calibrants.

**RESULTS**

**Screening Extracts for PC-containing Glycans**—In our previous work on ES-62, we showed that FAB-MS of perdeuterocetylated derivatives provides a sensitive means of detecting low molecular weight glycans substituted with PC (11). We have used a similar strategy in the present work to examine each of the parasite samples for PC-substituted glycans. N-Glycans were released by PNGase F digestion of reduced/carboxymethylated trypsinized material. The glycans were sepa-
The signals at 45 mass unit intervals below major signals in Fig. 1 correspond to under-deuteroacetylation.

| Signal (m/z) | Assignment |
|-------------|------------|
| 1311        | Hex_NAc<sub>2</sub>Na<sup>+</sup> |
| 1525        | FucHex_NAc<sub>4</sub>H<sup>+</sup> |
| 1547        | FucHex_NAc<sub>4</sub>Na<sup>+</sup> |
| 1586        | Hex_NAc<sub>4</sub>Na<sup>+</sup> |
| 1608        | Hex_NAc<sub>4</sub>Na<sup>+</sup> |
| 1822        | FucHex_NAc<sub>4</sub>Na<sup>+</sup> |
| 1844        | FucHex_NAc<sub>4</sub>Na<sup>+</sup> |
| 1879        | Hex_NAc<sub>4</sub>H<sup>+</sup> |
| 1901        | Hex_NAc<sub>4</sub>Na<sup>+</sup> |
| 1905        | Hex_NAc<sub>4</sub>Na<sup>+</sup> |
| 1999        | PCHex_NAc<sub>5</sub>H<sup>+</sup> |
| 2021        | PCHex_NAc<sub>5</sub>Na<sup>+</sup> |
| 2115        | FucHex_NAc<sub>5</sub>Na<sup>+</sup> |
| 2137        | FucHex_NAc<sub>5</sub>Na<sup>+</sup> |
| 2172        | Hex_NAc<sub>5</sub>H<sup>+</sup> |
| 2180        | Hex_NAc<sub>5</sub>Na<sup>+</sup> |
| 2194        | Hex_NAc<sub>5</sub>Na<sup>+</sup> |
| 2202        | Hex_NAc<sub>5</sub>Na<sup>+</sup> |
| 2235        | PCFucHexHexNAc<sub>6</sub>H<sup>+</sup> |
| 2257        | PCFucHexHexNAc<sub>6</sub>Na<sup>+</sup> |
| 2292        | PCHexHexNAc<sub>6</sub>H<sup>+</sup> |
| 2314        | PCHexHexNAc<sub>6</sub>Na<sup>+</sup> |
| 2408        | FucHexHexNAc<sub>6</sub>H<sup>+</sup> |
| 2430        | FucHexHexNAc<sub>6</sub>Na<sup>+</sup> |
| 2465        | HexHexNAc<sub>6</sub>Na<sup>+</sup> |
| 2487        | HexHexNAc<sub>6</sub>Na<sup>+</sup> |
| 2528        | PCFucHexHexNAc<sub>7</sub>H<sup>+</sup> |
| 2550        | PCFucHexHexNAc<sub>7</sub>Na<sup>+</sup> |
| 2585        | PCHexHexNAc<sub>7</sub>Na<sup>+</sup> |
| 2607        | FucHexHexNAc<sub>7</sub>Na<sup>+</sup> |
| 2723        | PCHexHexNAc<sub>7</sub>H<sup>+</sup> |
| 2821        | PCFucHexHexNAc<sub>8</sub>H<sup>+</sup> |
| 2843        | PCFucHexHexNAc<sub>8</sub>Na<sup>+</sup> |
| 2878        | PCHexHexNAc<sub>8</sub>H<sup>+</sup> |
| 2900        | PCHexHexNAc<sub>8</sub>Na<sup>+</sup> |
| 3114        | PCFucHexHexNAc<sub>9</sub>H<sup>+</sup> |
| 3136        | PCFucHexHexNAc<sub>9</sub>Na<sup>+</sup> |

The mass spectra of the PC-containing glycans from O. volvulus, PC-containing glycans from structures shown in Fig. 2. It is notable that the most abundant species are core-PC substitutions, such as PC. When we applied this strategy to the samples from ES-62 (11), a second portion of the glycans was treated with aqueous hydrofluoric acid using conditions that are known to cleave phosphodiester linkages. The products are permethylated and analyzed by MS. Molecular ions that are observed only after HF treatment are indicative of neutral N-glycans were observed in all three samples (Fig. 1 and Table I). Once again, these were consistent with data previously obtained from ES-62 (11).

Our earlier studies of ES-62 indicated that although glycans containing several PC substituents are rather intractable to direct MS analysis, putative glycan scaffolds for multiple PC substitution could be readily detected after PC release. We proposed the following simple strategy for selecting for large and/or multiply PC-substituted glycans (11). A portion of the sample is initially screened for the presence of components lacking PC moieties. This is achieved by MS analyses of permethylated derivatives using experimental procedures involving chloroform extraction that do not allow the recovery of charged material from the permethylation procedure. A second portion is then treated with aqueous hydrofluoric acid using conditions that are known to cleave phosphodiester linkages. The products are permethylated and analyzed by MS. Molecular ions that are observed only after HF treatment are indicative of neutral N-glycans with highly unusual antennae are present in all three nematodes.

**Figure 2. Proposed structures of the PC containing N-glycans of ES-62 (11).**

**Table I**

Assignments of molecular ions observed in FAB spectra of perdeuteroacetylated N-glycans of A. viteae, O. gibsoni, and O. volvulus eluting in 50% aqueous acetonitrile (v/v) fraction from a Sep-Pak C<sub>18</sub> cartridge.

| Signal (m/z) | Assignment |
|-------------|------------|
| 260         | HexNAc<sup>+</sup> |
| 464         | HexNAc<sup>-</sup> |
| 505         | HexNAc<sup>-</sup> |
| 679         | FucHexNAc<sup>-</sup> |
| 750         | HexNAc<sup>-</sup> |
| 782         | HexNAc<sup>-</sup> |
| 967         | HexHexNAc<sub>2</sub>Na<sup>-</sup> |
| 995         | HexNAc<sup>-</sup> |
| 1117        | HexHexNAc<sub>2</sub> |
| 1141        | FucHexHexNAc<sub>2</sub>Na<sup>-</sup> |
| 1171        | HexHexNAc<sub>2</sub>Na<sup>-</sup> |
| 1240        | HexNAc<sup>-</sup> |
| 1280        | HexNAc<sup>-</sup> |
| 1416        | HexHexNAc<sub>3</sub>Na<sup>-</sup> |
| 1579        | HexHexNAc<sub>3</sub>Na<sup>-</sup> |
| 1590        | FucHexHexNAc<sub>3</sub>Na<sup>-</sup> |
| 1661        | HexHexNAc<sub>3</sub>Na<sup>-</sup> |
| 1783        | HexHexNAc<sub>3</sub>Na<sup>-</sup> |
| 1835        | FucHexHexNAc<sub>3</sub>Na<sup>-</sup> |
| 1906        | HexHexNAc<sub>3</sub>Na<sup>-</sup> |
| 1987        | HexHexNAc<sub>3</sub>Na<sup>-</sup> |
| 2080        | FucHexHexNAc<sub>3</sub>Na<sup>-</sup> |
| 2101        | HexHexNAc<sub>3</sub>Na<sup>-</sup> |
| 2235        | FucHexHexNAc<sub>4</sub>Na<sup>-</sup> |
| 2396        | HexHexNAc<sub>4</sub>Na<sup>-</sup> |
| 2570        | FucHexHexNAc<sub>4</sub>Na<sup>-</sup> |
| 2641        | HexHexNAc<sub>4</sub>Na<sup>-</sup> |
| 2815        | FucHexHexNAc<sub>4</sub>Na<sup>-</sup> |
| 2886        | HexHexNAc<sub>4</sub>Na<sup>-</sup> |
| 3060        | FucHexHexNAc<sub>4</sub>Na<sup>-</sup> |
| 3314        | HexHexNAc<sub>4</sub>Na<sup>-</sup> |
| 3305        | FucHexHexNAc<sub>4</sub>Na<sup>-</sup> |
| 3376        | HexHexNAc<sub>4</sub>Na<sup>-</sup> |
| 3550        | FucHexHexNAc<sub>4</sub>Na<sup>-</sup> |
| 3621        | HexHexNAc<sub>4</sub>Na<sup>-</sup> |
| 3785        | FucHexHexNAc<sub>4</sub>Na<sup>-</sup> |
| 3866        | HexHexNAc<sub>4</sub>Na<sup>-</sup> |
| 4040        | FucHexHexNAc<sub>4</sub>Na<sup>-</sup> |
| 4111        | HexHexNAc<sub>4</sub>Na<sup>-</sup> |
| 4285        | FucHexHexNAc<sub>4</sub>Na<sup>-</sup> |
| 4356        | HexHexNAc<sub>4</sub>Na<sup>-</sup> |

**Table II**

Assignments of molecular and fragment ions observed in FAB and MALDI spectra of permethylated N-glycans of A. viteae, O. gibsoni, and O. volvulus after treatment with HF eluting in 50% aqueous acetonitrile (v/v) fraction from a Sep-Pak C<sub>18</sub> cartridge.

| Signal (m/z) | Assignment |
|-------------|------------|
| 2900        | PCHexHexNAc<sub>6</sub> |
| 2878        | PCHexHexNAc<sub>6</sub> |
| 3114        | FucHexHexNAc<sub>7</sub> |
| 3136        | FucHexHexNAc<sub>7</sub> |

Characterization of Novel Chito-oligomers—FAB-MS of...
FIG. 3. FAB mass spectrum above m/z 1400 of permethylated N-glycans from filarial nematodes. The N-glycans of A. viteae (A), O. gibsoni (B), and O. volvulus (C) were released from tryptic glycopeptides by digestion withPNGase F, separated from protein by Sep-Pak purification, treated with HF, and permethylated. The derivatized glycans were purified by Sep-Pak, and the 50% (v/v) aqueous acetonitrile fraction was screened by FAB-MS.

FIG. 4. MALDI mass spectrum above m/z 1400 of permethylated N-glycans from A. viteae. The N-glycans of A. viteae were released from tryptic glycopeptides by digestion with PNGase F, separated from protein by Sep-Pak purification, treated with HF, and permethylated. The derivatized glycans were purified by Sep-Pak, and the 50% (v/v) aqueous acetonitrile fraction was screened by MALDI-MS.
PNGase-F released permethylated glycans from the three species of filarial nematodes gave data corresponding to high mannose (Hex5–9HexNAc2) and truncated structures (Fuc0–1Hex2–4 HexNAc2) consistent with those previously observed in our studies of ES-62 (11) and were not investigated further (data not shown). In contrast, spectra from samples that had been treated with HF after the PNGase F digestion showed abundant new signals, the compositions of which suggested novel structures (Fig. 3). The spectra are dominated by a series of glycans with composition HexNAc3–11Hex3Fuc0–1 (Table II). These compositions are consistent with substoichiometrically fucosylated trimannosyl cores to which are added between one and eight N-acetylhexosamine residues. As previously observed (Fig. 1 and Table I), the N-glycans of *O. volvulus* are core-fucosylated to a much lesser degree than those of *A. viteae* and *O. gibsoni*.

In order to ensure that the data acquired after HF treatment were associated with N-glycans released by PNGase F digestion, rather than being derived from saccharides present in the original sample, the following series of experiments was carried out. Worm material was reduced, carboxymethylated, and digested with trypsin, and the product mixture was fractionated on reverse phase Sep-Pak C18. The aqueous eluent, which would contain oligo- and polysaccharide components, was analyzed in the same way as described above for PNGase F-released glycans, whereas the included fractions containing peptides and glycopeptides was subjected to PNGase F digestion as before. These experiments (data not shown) confirmed

Table III

| Elution time | Characteristic fragment ions | Assignment          | Relative abundance |
|--------------|------------------------------|---------------------|--------------------|
| 16.85        | 115, 118, 131, 162, 175      | Terminal fucose     | 0.21               |
| 18.55        | 102, 118, 129, 145, 161, 205 | Terminal mannose    | 0.44               |
| 18.83        | 102, 118, 129, 145, 161, 205 | Terminal galactose  | 0.01               |
| 19.78        | 129, 130, 161, 190           | 2-Linked mannose    | 0.65               |
| 20.23        | 102, 118, 129, 162, 189, 233 | 6-Linked mannose    | 0.07               |
| 21.02        | 130, 190, 233                | 2,4-Linked mannose  | 0.17               |
| 21.45        | 129, 130, 189, 190           | 2,6-Linked mannose  | 0.09               |
| 21.62        | 118, 129, 189, 234           | 3,8-Linked mannose  | 0.51               |
| 22.62        | 117, 159, 203, 205           | Terminal GlcNAc     | 0.28               |
| 23.10        | 117, 159, 203, 205           | Terminal GalNAc     | 0.04               |
| 23.57        | 117, 159, 233                | 4-Linked GlcNAc     | 1.00               |
| 24.92        | 117, 159, 261                | 4,6-Linked GlcNAc   | 0.06               |
FIG. 6. Positive ion CAD mass spectrum of the *A. viteae* HexNAc$_5$ A-type fragment ion. Structurally informative fragment ions are assigned on the inset. The fragment ions at *m/z* 154 and 196 are a ketene increment apart. The latter has a mass equivalent to loss of two methanol groups from *m/z* 260 and is probably formed by extrusion of HexNAc moieties from the parent ion by a combination of elimination and glycosidic cleavage.

FIG. 7. GC-MS linkage analysis of PNGase F released N-glycans of *A. viteae*. The part of the chromatogram showing variously linked HexNAc residues is shown as the total ion current (bottom panel) and selected ion monitoring of HexNAc specific fragment ions 117 (middle panel) and 159 (top panel).
that the chito-oligomeric components were not present in the aqueous eluent and were only observed after PNGase F digestion of the glycopeptide fraction.

Corroborative evidence for the existence of a family of N-acetylated hexosamine-rich N-glycans was provided by MALDI-MS, which yields molecular ions at very high sensitivity (Fig. 4). In addition to the molecular ions consistent with compositions HexNAC\(_{3-11}\)Hex\(_2\)Fuc\(_0-1\), the added sensitivity of the instrumentation allows the detection of larger structures in the series up to a maximum composition of HexNAC\(_{15}\)Hex\(_3\).

An important feature of FAB-MS is that it yields abundant fragment ions (A-type fragment ions), which are formed by cleavages at HexNAC residues. These provide information on the types and lengths of antennae (21, 22). The low mass fragment ion region of the FAB spectra were very similar for all three filamentous nematodes; the A. viteae spectrum is shown as a representative example (Fig. 5 and Table II). The most abundant A-type ions occur at \(m/z\) 260, 505, 750, 995, and 1240, which correspond to HexNAC\(_{1-5}\). This indicates the presence of N-glycans with highly unusual antennae comprising up to five N-acetylated hexosamine residues; in some experiments, an additional A-type ion at \(m/z\) 1485 was observed, indicating the probable presence of HexNAC\(_{5}\) as a minor constituent. To further explore the nature of the unusual HexNAC-rich antenna, the A-type fragment ions were subjected to CAD MS-MS experiments. The CAD MS-MS spectrum of HexNAC\(_{3}\) (\(m/z\) 1240 (Fig. 6)) contains three series of daughter ions (\(m/z\) 995, 750, 505, and 260; \(m/z\) 1009, 764, 519, and 274; and \(m/z\) 1178, 933, 688, 443, and 198), the derivation of which is shown in Fig. 6, inset. These fragment ions are consistent with an unbranched sequence of five N-acetylated hexosamine residues in 1–4 linkage.

**Linkage Analysis of HF-treated Glycans—** Linkage analysis data for the HF-treated N-glycans of A. viteae are shown in Table III. The other two species of filarial nematode gave similar data. From these data we can conclude that the presence of high levels of 2-Man, 2,4-Man, and 2,6-Man is consistent with bi-, tri-, and tetraantennary structures. These structures contain highly unusual chito-oligomeric antennae comprising up to five \(\beta\)-linked GlcNAc residues capped with GlcNAc or GalNAc (Fig. 8).

**DISCUSSION**

Obtaining levels of *O. volvulus* PC-glycans amenable to structural analysis is extremely difficult due to problems in getting sufficient parasites (adult *O. volvulus* specimens are only obtainable from infected humans in the tropics) to collect secreted proteins. We approached this problem by preparing a whole worm extract (only a few parasites are required for this), as analysis of *A. viteae* showed conservation of PC-glycan structure between ES-62 and components of whole worm extracts. We also obtained secretions from the more readily available and virtually antigenically indistinguishable (13) bovine parasite, *O. gibsoni*. These experiments allowed us to come to the important conclusion that both *Onchocerca* spp. have PC-glycans that are similar to those found on ES-62 and *A. viteae* as a whole. This validated employing the laboratory-based rodent parasite in research aimed at designing new drugs for combating *O. volvulus*.

In addition to the PC-glycans described above, the analysis employing HF also revealed the existence of a second family of N-glycans that are remarkably rich in GlcNAc. It has not yet been defined whether these contain FC, or whether some other polar group is being released by the HF (experiments to address this issue are in progress); if the former is the case, then it is probable that, as with the PC-glycans characterized to date, attachment of PC is likely to be to GlcNAc. This would suggest that identical biosynthetic machinery would be used in PC attachment in both cases, an important consideration in relation to drug development. The second group of N-glycans have highly unusual chito-oligomeric antennae containing up to five (possibly six) GlcNAc residues (Fig. 8). Although GlcNAc-capped chitobiase has previously been observed as a very minor antenna in N-glycans from the serine protease of pit viper venom (24), this is the first report of chito-oligomers in a eukaryotic glycoprotein. The closest homology to these structures in nature are the Nod factors, which are signal molecules produced by *Azorhizobium*, *Bradyrhizobium*, and *Rhizobium* species that trigger nodule formation in leguminous plants (25). Recently it has been shown that Nod-like molecules are...
likely to play important roles in vertebrate development. Thus the *Xenopus* protein DG42, which is expressed for a short time during embryo development, has been shown to synthesize chito-oligomers in *in vitro* experiments (26). Evidence for a developmental role of short chitin oligosaccharides in vertebrate development has recently been obtained by Bakkers et al. (27), who demonstrated that zebrafish fertilized eggs injected with antiserum against the DG42 protein led to embryos with severe defects in trunk and tail development. It is reasonable to speculate that vertebrates have lectin-like receptors for any chito-oligomers that are playing roles in development. Thus, it is possible that putative host receptors for such oligomers might play a role in parasite-host interactions. Clearly much more needs to be done to establish whether chito-oligomers have a role in signaling mechanisms in vertebrates. Nevertheless the data in this paper, which provide the first direct evidence for the existence of chito-oligomers in eukaryotic glycoproteins, are an important step forward in this area of research.

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REFERENCES
1. Harnett, W., and Parkhouse, R. M. E. (1995) in *Perspectives in Nematode Physiology and Biochemistry* (Sood, M. L., and Kapur, J., eds) pp. 207–242, Narendra Publishing House, Delhi, India
2. Lal, R. B., Kumaraswami, V., Steel, C., and Nutman, T. B. (1990) *Am. J. Trop. Med. Hyg.* 42, 56–64
3. Harnett, W., and Harnett, M. M. (1993) *J. Immunol.* 151, 4829–4837
4. Deehan, M. R., Harnett, M. M., and Harnett, W. (1997) *J. Immunol.* 159, 6105–6111