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Structural Analysis of *N*-Linked Glycans in *Caenorhabditis elegans*[1]

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***Caenorhabditis elegans* is an excellent model for morphogenetic research. However, little information is available on the structure of cell-surface glycans in *C. elegans*, although several lines of evidence have suggested a role for these glycans in cell–cell interactions during development. In this study, we analyzed *N*-glycan structures. Oligosaccharides liberated by hydrazinolysis from a total membrane fraction were labeled by pyridylamination, and around 90% of the *N*-glycans were detected as neutral oligosaccharides. The most dominant structure was Manα1-6(Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc, which is commonly found in insects. Branching structures of major oligomannose-type glycans were the same as those found in mammals. Structures that had a core fucose or non-reducing end *N*-acetylglucosamine were also identified, but ordinary complex-type glycans with *N*-acetyllactosamine were not detected as major components.**

Key words: *Caenorhabditis elegans*, mass spectrometry, *N*-glycans, oligomannose-type, pyridylamination.

N-Glycans linked to the asparagine residues of proteins are widely distributed in eukaryotes. Unicellular eukaryotes have *N*-glycans elongated by oligo- or poly-saccharides with relatively simple components. All other eukaryotes, multicellular organisms, have acquired complex-type *N*-glycans consisting of various components. The complex-type *N*-glycans were probably first expressed when multicellular organisms appeared in order to mediate the cell–cell and cell–matrix interactions essential for development (*1*). Indeed, several genes involved in the biosynthesis of complex-type *N*-glycans have already been identified in one of the simplest multicellular organisms, *Caenorhabditis elegans* (*2*). GlcNAc-TI, a key enzyme in the formation of complex-type *N*-glycans, is essential for normal embryogenesis in *C. elegans* (*3*) and mice (*4, 5*), although culture cell lines with mutations in the gene for GlcNAc-TI show no change in phenotype. This finding indicates that these glycans are not required for the growth of cultured cells but are indispensable for normal morphogenesis.

Metazoa are classified into two major groups, deuterostomes and protostomes. Much structural information is available on *N*-glycans of the former group, since it includes vertebrates. However, information on the latter group has mainly been procured from insect cells utilized for the expression of recombinant proteins. The genome project for the protostome *C. elegans* has revealed that this nematode has a set of genes for biosynthetic enzymes forming *N*-glycans with several exceptions, for example, sialyltransferases and GlcNAc-TIII. However, information on *N*-glycan structures in *C. elegans* is scarce. Only preliminary reports had been presented (*6, 7*). In this paper, we report on the structural elucidation of *N*-linked oligosaccharides from *C. elegans*.

MATERIALS AND METHODS

Standard PA-Oligosaccharides—The structures and abbreviations of the authentic PA-glycans used in this study are listed in Table I. M3GN-1 and M3GN-2 were purchased from Takara Biomedicals. All the others were prepared as described previously (*8*).

Preparation of Crude Membrane Fraction from C. elegans—Mixed cultures of *C. elegans* (Bristol N2) grown in liquid media were collected by centrifugation, then washed with M9 buffer to separate the baits (*9*). Worms were homogenized by sonication in five volumes of 10 mM EDTA, pH 8.0. After removal of soluble materials by centrifugation at 17,000 ×*g*, precipitates were washed with 10 mM EDTA, pH 8.0, then collected by re-centrifugation. The precipitate, delipidated by sonication in acetone, was dried, and used for *N*-glycan analysis as a crude membrane fraction.

Preparation of Pyridylaminated Glycans—Glycans were liberated from glycoproteins by hydrazinolysis as described previously (*10*). Briefly, 20 mg of the crude membrane fraction was heated at 100°C for 10 h with 2 ml of anhydrous hydrazine. After the hydrazine had been evaporated off, *N*-acetylation was initiated with acetic anhydride in a satu-

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Abbreviations: dHex, deoxyhexose; GlcNAc, *N*-acetyl-D-glucosamine; GlcNAcase, *N*-acetylglucosaminidase; GlcNAc-TI, *N*-acetylglucosaminyltransferase-I; GlcNAc-TII, *N*-acetylglucosaminyltransferase-II; GlcNAc-TIII, *N*-acetylglucosaminyltransferase-III; GN2, pyridylamino-*N,N'*-diacetylchitobiose; Hex, hexose; HexNAc, *N*-acetylhexosamine; MALDI-TOF-MS, matrix assisted laser desorption–time of flight–mass spectrometry; Man, D-mannose; PA-, pyridylamino.

TABLE I. **Structures and elution positions in HPLC of standard PA-oligosaccharides.**

Abbreviation	Structure	Elution position in HPLC	
		RP (Gu)	Size (Gu)
M2A	Manα1-6(Manβ1-4GlcNAcβ1-4GlcNAc-PA)-3	5.75	3.51
M2B	Manα1-3(Manβ1-4GlcNAcβ1-4GlcNAc-PA)-6	6.81	3.51
M3B	Manα1-6(Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-PA	6.80	4.54
$M3BF_6$	Fucα1-6, Manα1-6(Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-PA	8.48	4.95
M3GN-1	GlcNAcβ1-2Manα1-3(Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAc-PA	6.63	5.42
M3GN-2	GlcNAcβ1-2Manα1-6(Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-PA	8.68	5.42
M5A	Manα1-6(Manα1-3)Manα1-6(Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-PA	6.92	6.51
M6B	Manα1-6(Manα1-3)Manα1-6(Manα1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-PA	6.51	7.47
M7A	Manα1-2Manα1-6(Manα1-3)Manα1-6(Manα1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-PA	5.66	8.42
M8A	Manα1-2Manα1-6(Manα1-3)Manα1-6(Manα1-2Manα1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-PA	5.36	9.26
M9A	Manα1-2Manα1-6(Manα1-3)Manα1-6(Manα1-2Manα1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-PA	5.74	9.97
GM9	Manα1-2Manα1-6(Manα1-2Manα1-3)Manα1-6(Glcα1-3Manα1-2Manα1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-PA	7.29	10.91

rated sodium bicarbonate solution. The glycans were passed through a cation exchanger, Dowex 50W ×2 (H^+), to remove sodium ions. Reducing ends of the liberated glycans were tagged with a fluorophore, 2-aminopyridine. Lyophilized samples were heated at 90°C for 60 min with 200 μl of a pyridylamination reagent described elsewhere (*10*), and after addition of 700 μl of reducing reagent, heated at 80°C for 35 min. Excess reagent was removed by chloroform extraction with ammonia alkali solution. After the evaporation of ammonia, the PA-glycans were separated on a TSK-gel HW-40F column (1.5 × 48 cm) equilibrated with 10 mM ammonium acetate, pH 6.0. Fractions of 8 ml were collected, and the fluorescence of each fraction was measured with a fluorescence spectrophotometer with excitation at 320 nm and emission at 400 nm. The fraction containing PA-*N*-glycans was further purified with a Sep-Pak Plus C_{18} cartridge (Waters, MA) as described before (*11*). The PA-glycans adsorbed by the resin were eluted with 2 ml of 20% methanol in 0.1% acetic acid solution.

Reducing End-Monosaccharide Analysis—PA-Glycans were hydrolyzed with 6 M hydrochloric acid at 100°C for 4 h. After complete evaporation of the hydrochloride, liberated monosaccharides were re-*N*-acetylated by acetic anhydride in saturated sodium bicarbonate solution. PA-monosaccharides derived from reducing ends were purified on a small cation exchange column as described previously (*12*). Briefly, the sample was applied to a Dowex 50W ×2 (H^+) column (0.5 × 3 cm). The column was washed with 2 ml of water, then PA-sugars were eluted with 3 ml of 2.5% ammonia solution. The eluate was lyophilized to remove ammonia, then analyzed by HPLC (*13*). PA-sugars were separated on a TSKgel Sugar AX-I column (0.46 × 15 cm) equilibrated with 10% acetonitrile in 0.8 M potassium borate, pH 9.0 at a flow rate of 0.3 ml/min at 74°C, and detected with a fluorescence spectrophotometer at an excitation wavelength of 310 nm and emission wavelength of 380 nm.

High Performance Liquid Chromatography for PA-Glycan Separation—Size-fractionation HPLC was performed on a Shodex NH2P-50 column (0.46 × 15 cm, Showa Denko) at a flow rate of 0.8 ml/min. The column was equilibrated with 50 mM ammonium acetate, pH 7.0 containing 86.5%

acetonitrile. After a sample had been injected, the acetonitrile concentration was decreased linearly from 86.5 to 37.5% in 50 min. The PA-glycans were detected with a fluorescence spectrophotometer at 310 nm excitation and 380 nm emission. The molecular size of each PA-glycan is given in glucose units based on the elution times of the PA-isomaltooligosaccharides. Reversed phase HPLC was performed on a Cosmosil 5C18-P column (0.46 × 15 cm, Nacalai Tesque) at a flow rate of 1.5 ml/min. The column was equilibrated with 100 mM ammonium acetate, pH 4.0, containing 0.025% 1-butanol. After injection of a sample, the 1-butanol concentration was increased linearly from 0.025 to 0.5% in 55 min. The PA-glycans were detected at 315 nm excitation and 400 nm emission. The retention time of each PA-glycan is given in glucose units based on the elution times of the PA-isomaltooligosaccharides. The behavior of authentic PA-glycans on HPLC is shown in Table I.

Glycosidase Digestion—PA-oligosaccharides were digested with bovine kidney α-fucosidase (Sigma) in 100 mM sodium phosphate buffer, pH 5.5, for 16 h at 37°C. The enzyme concentration was 10 units/ml for digestion of both α-1,3- and α-1,6-linkages, and 1 unit/ml for α-1,6-linkages alone. The substrate specificity of this enzyme was described before (*14*).

PA-oligosaccharides were digested with 0.5 unit of Jack bean α-mannosidase (Seikagaku Kogyo) in 50 mM sodium citrate buffer, pH 4.5, for 20 h at 37°C, 0.1 unit of *Achatina fulica* β-mannosidase (Seikagaku Kogyo) in 50 mM sodium citrate buffer, pH 4.5, for 40 h at 37°C, or 0.1 unit of Jack bean β-*N*-acetylhexosaminidase (Seikagaku Kogyo) in 100 mM sodium citrate buffer, pH 5.0, for 20 h at 37°C.

Mass Spectrometric Analysis—PA-Glycans were co-crystallized in a matrix of 2,5-dihydroxybenzoic acid. Mass spectra were recorded using a Voyager-DE-RP BioSpectrometry Workstation (Perseptive Biosystems, MA) with delayed extraction operated in the reflector mode.

RESULTS

Preparation of N-Glycans from C. elegans—*N*-Glycans were liberated from the crude membrane fraction of *C. elegans* by hydrazinolysis and re-*N*-acetylation. To detect sugars with high sensitivity, the reducing ends of the glycans were tagged with a fluorophore, 2-aminopyridine. The pyridylaminated glycans were isolated from byproducts and excess reagent by gel filtration. The fraction containing *N*-glycan was detected by reducing end-monosaccharide analysis (Fig. 1). As the reducing end of *N*-glycan was GlcNAc, the aliquot (Fractions 4–6) of gel filtrate that contained the reducing end PA-GlcNAc was collected as the *N*-glycan

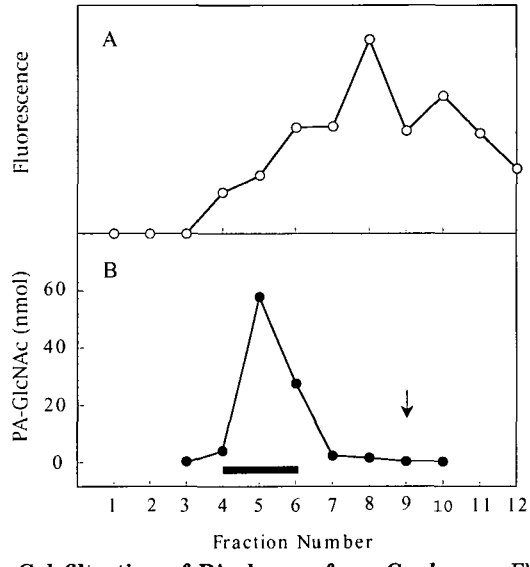

Fig. 1. **Gel filtration of PA-glycans from *C. elegans*.** Fluorescence-tagged glycans were purified by removal of by-products and excess reagents. Fluorescence intensity (A) and reducing end GlcNAc content (B) of the fractions are shown. Arrow indicates the elution position of PA-monosaccharide.

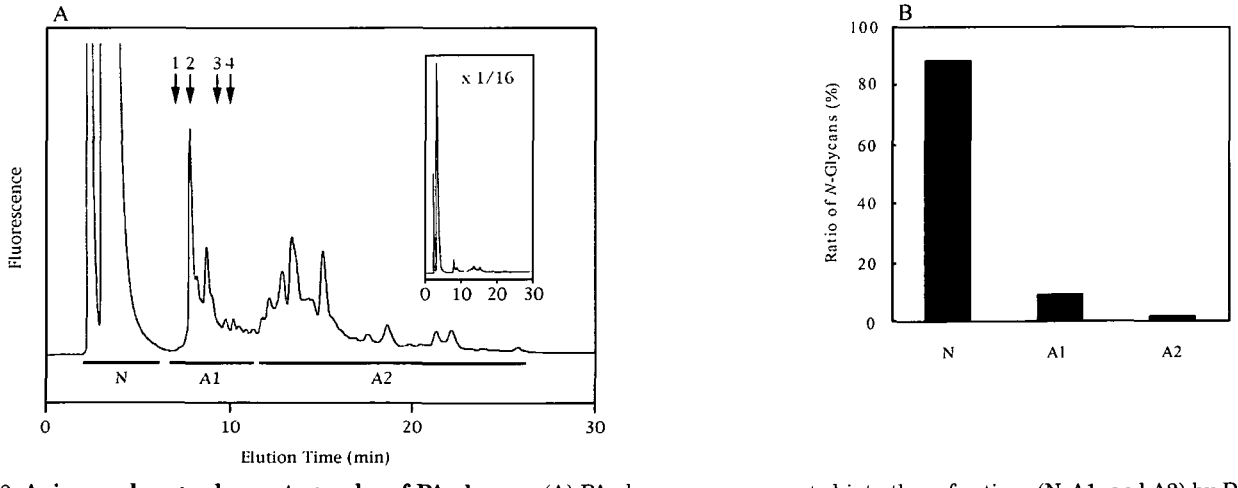

Fig. 2. **Anion-exchange chromatography of PA-glycans.** (A) PA-glycans were separated into three fractions (N, A1, and A2) by DEAE-HPLC. Numbered arrows indicate the elution positions of standard *N*-glycans with the corresponding number of sialic acid residues. Inset shows the reduced chromatogram profile at a magnification of 1/16. (B) Ratio of *N*-glycans in each fraction. Analytical method is described in "MATERIALS AND METHODS."

fraction. This was further purified with a Sep-Pak C_{18} cartridge.

Separation of N-Glycans by DEAE-HPLC—PA-*N*-glycans were separated by DEAE-anion-exchange chromatography to obtain neutral (N) and acidic (A1 and A2) *N*-glycan fractions (Fig. 2A). Contents of the *N*-glycans in the fractions were measured as amounts of reducing end PA-GlcNAc (Fig. 2B). About 90% of *N*-glycans were detected in the neutral fraction (N), 9% in the low acidic fraction (A1), and 2% in the highly acidic fraction (A2).

Structure Analysis of Neutral N-Glycans—The neutral PA-*N*-glycans were analyzed by size-fractionation HPLC, in which separation depended on the molecular size of the oligosaccharides. Eleven major peaks (G0–G10) were fractionated (Fig. 3), each of which was further purified by reversed phase HPLC. Peaks G2 and G3 were separated into two major components each, designated G2-1, G2-2, G3-1, and G3-2, in reversed phase HPLC. The peak marked by the asterisk in Fig. 3 did not contain much PA-oligosaccharide according to the results of reversed phase HPLC. Elution positions of G0–G10 on size-fractionation and reversed phase HPLC are summarized in Fig. 4 as a two-dimen-

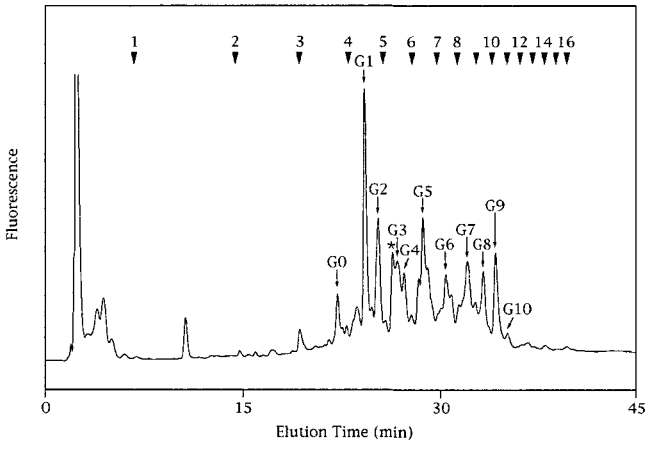

Fig. 3. **Size-fractionation HPLC of neutral PA-*N*-glycans.** Eleven major peaks larger in molecular size than isomaltotriose are represented as G0–G10. Numbered arrowheads indicate the elution positions of PA-isomaltooligosaccharides with the corresponding degree of polymerization.

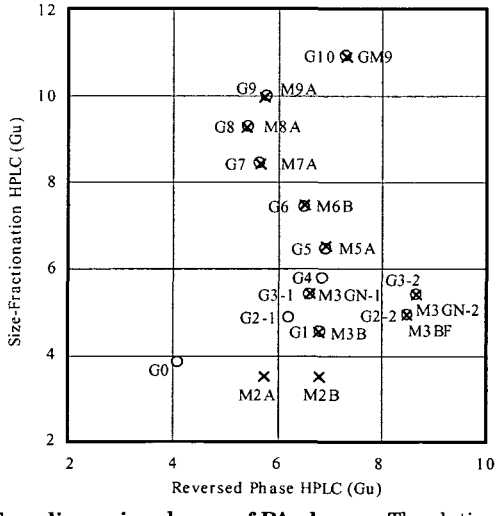

Fig. 4. **Two-dimensional map of PA-glycans.** The elution profiles of each PA-glycan on size-fractionation and reversed phase HPLC were expressed in glucose units (Gu) based on the elution times of the PA-isomaltooligosaccharides and plotted on the map. Circles indicate the positions of the PA-glycans from *C. elegans*. X indicates the positions of the standard PA-glycans.

TABLE II. **Mass analysis of PA-glycans from *C. elegans*.**

Fraction	Mass (observed)	Mass (expected)	Estimated composition
G1	989.2 1011.2 1027.2	989.39 (H^+) 1011.38 (Na^+) 1027.48 (K^+)	$Hex_3HexNAc_2$-PA
G2-1	1119.1 1141.1 1157.3	1119.46 (H^+) 1141.44 (Na^+) 1157.55 (K^+)	$dHex_2Hex_2HexNAc_2$-PA
G2-2	1134.8 1156.9 1172.8	1135.45 (H^+) 1157.43 (Na^+) 1173.54 (K^+)	$dHex_1Hex_3HexNAc_2$-PA
G3-1	1191.8 1213.9 1229.9	1192.47 (H^+) 1214.42 (Na^+) 1230.56 (K^+)	$Hex_3HexNAc_3$-PA
G3-2	1214.6 1230.5	1214.42 (Na^+) 1230.56 (K^+)	$Hex_3HexNAc_3$-PA
G4	1281.4 1303.4 1319.4	1281.51 (H^+) 1303.49 (Na^+) 1319.60 (K^+)	$dHex_2Hex_3HexNAc_2$-PA
G5	1313.0 1334.9 1350.9	1313.50 (H^+) 1335.48 (Na^+) 1351.59 (K^+)	$Hex_5HexNAc_2$-PA
G6	1497.3 1513.2	1497.53 (Na^+) 1513.64 (K^+)	$Hex_6HexNAc_2$-PA
G7	1636.5 1658.5 1674.4	1637.60 (H^+) 1659.59 (Na^+) 1675.60 (K^+)	$Hex_7HexNAc_2$-PA
G8	1799.1 1821.2 1837.0	1799.66 (H^+) 1821.64 (Na^+) 1837.75 (K^+)	$Hex_8HexNAc_2$-PA
G9	1961.1	1961.71 (H^+)	$Hex_9HexNAc_2$-PA
G10	2123.6 2145.4 2161.3	2123.76 (H^+) 2145.75 (Na^+) 2161.85 (K^+)	$Hex_{10}HexNAc_2$-PA

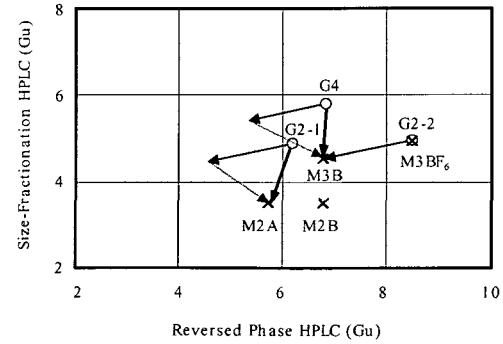

Fig. 5. **Alpha-fucosidase digestion of PA-glycans.** Circles indicate the positions of the PA-glycans from *C. elegans*. X marks the positions of the standard PA-glycans. Thin arrows indicate the direction of the changes in the elution positions after digestion with a low concentration of α-fucosidase, which can liberate α1-6 linked fucose at the *N*-glycan core. Thick arrows are the direction of the changes in the elution positions after digestion with a high concentration of α-fucosidase. Dotted arrows are the estimated contribution of the liberation of α-1,3-fucose.

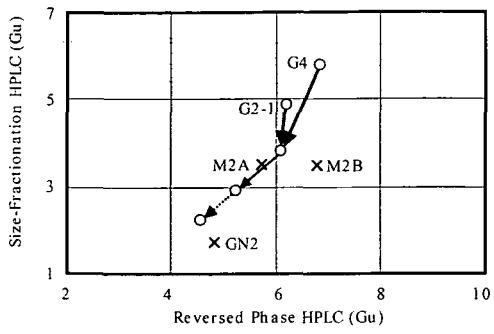

Fig. 6. **Glycosidase digestions of G2-1.** Circles indicate the positions of G2-1 and its digests. Thick arrows indicate the direction of the changes in the elution positions after digestion with α-mannosidase. The thin arrow is the direction of the changes in the elution positions after digestion with β-mannosidase. The dotted arrow is the direction of the changes in the elution positions after digestion with β-*N*-acetylhexosaminidase. X marks the positions of the standard PA-glycans.

sional map. From the positions on the map corresponding to standard PA-*N*-glycans, G1, G2-2, G3-1, G3-2, G5, G6, G7, G8, G9, G10 are estimated to be M3B, $M3BF_6$, M3GN-1, M3GN-2, M5A, M6B, M7A, M8A, M9A, and GM9, respectively. These structures were confirmed by mass analysis (Table II). G2-2 was susceptible to a low concentration of bovine kidney α-fucosidase, and the digest corresponded to M3B on the map (Fig. 5). These results also supported the conclusion that G2-2 has the structure $M3BF_6$. The results of reducing end analysis and mass spectrometry indicated that G0 was not an *N*-glycan (data not shown). Mass analysis suggested that G2-1 and G4 have two deoxyhexose residues (Table II). Digestion with a low concentration of α-fucosidase liberated a single fucose residue from G2-1 and G4. These contributions to the positions on the map are the same as those for fucosidase digestion of G2-2 (thin arrows in Fig. 5). From the additivity rule for the PA-glycans on reversed phase HPLC (*15, 16*), it is suggested that G2-1, G2-2, and G4 have a fucose residue at the same site, position 6 of the proximal GlcNAc in the *N,N'*-diacetylchitobi-

TABLE III. **Estimated structures of PA-*N*-glycans from *C. elegans*.**

Fraction	Estimated structure	Abbreviation	Ratio
G1	Manα1-(6)-Manα1-(3)-Manβ1-4GlcNAcβ1-4GlcNAc-PA	M3B	(100)[a]
G2-1	Manα1-(6)-Manβ1-4GlcNAcβ1-4GlcNAc-PA; Fucα1-(6); Fucα1-(3)	$M2AF_3F_6$	24
G2-2	Manα1-(6)-Manα1-(3)-Manβ1-4GlcNAcβ1-4GlcNAc-PA; Fucα1-(6)	$M3BF_6$	31
G3-1	Manα1-(6)-Manβ1-4GlcNAcβ1-4GlcNAc-PA; GlcNAcβ1-2Manα1-(3)	M3GN-1	11
G3-2	GlcNAcβ1-2Manα1-(6)-Manβ1-4GlcNAcβ1-4GlcNAc-PA; Manα1-(3)	M3GN-2	5
G4	Manα1-(6)-Manβ1-4GlcNAcβ1-4GlcNAc-PA; Fucα1-(6); Manα1-(3); Fucα1-(3)	$M3BF_3F_6$	12
G5	Manα1-(6)-Manα1-(3)-Manα1-(6)-Manβ1-4GlcNAcβ1-4GlcNAc-PA; Manα1-(3)	M5A	46
G6	Manα1-(6)-Manα1-(3)-Manα1-(6)-Manβ1-4GlcNAcβ1-4GlcNAc-PA; Manα1-2Manα1-(3)	M6B	22
G7	Manα1-2Manα1-(6)-Manα1-(3)-Manα1-(6)-Manβ1-4GlcNAcβ1-4GlcNAc-PA; Manα1-2Manα1-(3)	M7A	15
G8	Manα1-2Manα1-(6)-Manα1-(3)-Manα1-(6)-Manβ1-4GlcNAcβ1-4GlcNAc-PA; Manα1-2Manα1-2Manα1-(3)	M8A	19
G9	Manα1-2Manα1-(6)-Manα1-(3)-Manα1-(6)-Manβ1-4GlcNAcβ1-4GlcNAc-PA; Manα1-2Manα1-2Manα1-(3)	M9A	34
G10	Manα1-2Manα1-(6)-Manα1-2Manα1-(3)-Manα1-(6)-Manβ1-4GlcNAcβ1-4GlcNAc-PA; Glcα1-3Manα1-2Manα1-2Manα1-(3)	GM9	3

[a]Ratios were calculated from the peak areas on reversed phase HPLC. The peak area of G1 was taken as 100.

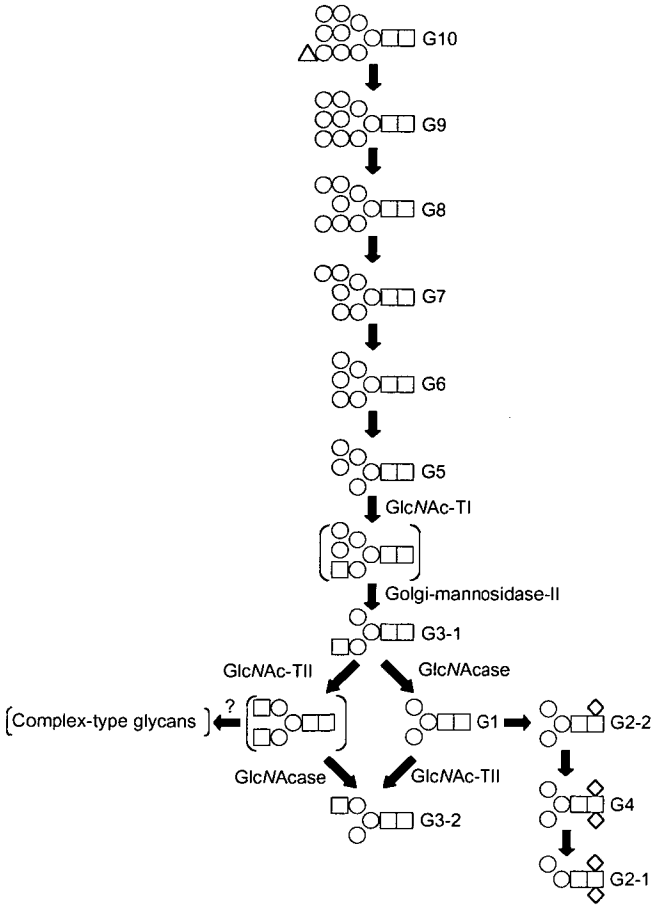

Fig. 7. **Hypothetical biosynthetic pathway of *C. elegans* *N*-glycans.** The structures of the glycans are shown by symbols. Circles, triangle, squares, and diamonds indicate mannoses, glucose, *N*-acetylglucosamines, and fucoses, respectively. The structures in parenthesis were not detected in this study.

ose core. Digestion with a high concentration of α-fucosidase liberated another fucose (thick arrows in Fig. 5). The positions of the digests from G2-1 and G4 coincide with those of M2A and M3B, respectively. The contribution of the second fucose of G2-1 and G4 (dotted arrows in Fig. 5) is similar to that of α-1,3-linked-fucose at proximal GlcNAc reported before (*17*). These lines of evidence suggest that the structures of G2-1 and G4 are $M2AF_3F_6$ and $M3BF_3F_6$, respectively. The structure of G2-1 was confirmed by another glycosidase digestion experiment. G2-1 was sequentially digested with α-mannosidase, β-mannosidase, and β-*N*-acetylhexosaminidase. Each digestion released one saccharide, and finally an oligosaccharide of 2.26 glucose units on size-fractionation HPLC (Fig. 6) was formed that corresponded to GlcNAc-PA with two fucoses. This result excludes the possibility that two fucoses linked to a saccharide other than proximal GlcNAc. Alpha-mannosidase digestion released two mannoses from G4, and the position of the digest on the map coincided with that of the α-mannosidase-digest of G2-1. This result confirms that G4 has the same core structure as G2-1. The estimated structures of neutral PA-*N*-glycans from *C. elegans* are summarized in Table III.

DISCUSSION

Glycan-mapping, mass spectrometry and glycosidase digestion revealed the structures of major *N*-glycans in *C. elegans*, an organism which serves as an excellent model for glycobiology. A hypothetical biosynthetic pathway of these *N*-glycans is illustrated in Fig. 7. Included were oligomannose-type glycans (G5–G10) with the same branching isomers as those in mammals. This may indicate a similarity of substrate specificity between *C. elegans* and mammalian α-1,2-mannosidases. Although there are seven candidate genes for class I α-mannosidase in *C. elegans*, no genes for ER and Golgi-mannosidase-I have been identified.

A typical paucimannose-type glycan, M3B (G1), was the most abundant component of the *C. elegans* *N*-glycans. This structure is also dominant in insects, which, like *C. elegans*, belong to the protostomes, and a biosynthetic pathway of the M3B structure in insect cells has been proposed. Because of the limited substrate specificity of Golgi-mannosidase-II, this pathway requires a Golgi-resident *N*-acetylglucosaminidase to make the M3B structure. Indeed, a membrane-bound *N*-acetylglucosaminidase which could hydrolyze M3GN-1 has been reported as a candidate (*18*). It has also been reported that an inhibitor of *N*-acetylglucosaminidase can increase elongated complex-type *N*-glycans in cultured insect cells (*19*). This phenomenon suggests the participation of *N*-acetylglucosaminidase in the biosynthesis of the truncated, namely, paucimannose-type, *N*-glycans. The abundance of M3B demonstrated in this study strongly suggests that *C. elegans* also possesses a processing *N*-acetylglucosaminidase, though the gene for it has yet to be discovered.

Other paucimannose-type glycans were also detected in this study (G2-1, G2-2, and G4). They have one or two fucoses attached at the innermost GlcNAc *via* an α-1,3- or α-1,6-linkage. Such structures have already been found in an insect glycoprotein, honeybee venom phospholipase A_2 (*14*). The snail *Lymnaea stagnalis*, another protostome, has been reported to possess α-1,3-fucosyltransferase activity to transfer a fucose to the core GlcNAc (*20*). However, an α-1,3-linked-fucose at the core of *N*-glycans has not been found in deuterostomes, while the core-α-1,6-linked fucose is common in animals, namely, metazoans. Since plants also have the core α-1,3-fucose, deuterostomes probably lost an active core-α-1,3-fucosyltransferase gene during their evolution.

Some *N*-glycans were elongated by GlcNAc at non-reducing terminals (G3-1 and G3-2). They appear to be precursors of ordinary complex-type *N*-glycans synthesized by GlcNAc-TI or GlcNAc-TII. These glycan structures support the notion that GlcNAc-TI and GlcNAc-TII are actually functional in *C. elegans* cells. Since disruption of the GlcNAc-TI gene causes embryonic lethality in *C. elegans* (*3*), as well as in mice (*4, 5*), the finding of the glycan produced by GlcNAc-TI is interesting for functional studies of *N*-glycans. Although the *N*-acetyllactosamine structure is a common backbone of various glycans in vertebrates, it was not detectable in the major *N*-glycans of *C. elegans*. A dozen minor *N*-glycans from *C. elegans* were also analyzed by mass spectrometry, but none of them showed any possibility of containing the *N*-acetyllactosamine structure (data not shown). We mention here three possible reasons for the

absence of such a structure. First, expression of the ordinary complex-type *N*-glycans may be limited to small spaces and/or short periods during the development of *C. elegans*. In this case, it is reasonable that such glycans could not be detected, because their levels would be extremely low. Second, *C. elegans* may have alternative structures without *N*-acetyllactosamine and without a synthetic pathway corresponding to the vertebrate complex-type *N*-glycan system. Some minor components whose structures have not been determined in this study may be candidates for alternative glycans of the new category. Third, both of these reasons may apply.

The observations reported here clearly indicate that the nematode *C. elegans* has the *N*-glycans commonly found in insects. The amount of ordinary complex-type *N*-glycans differs remarkably between *C. elegans* and vertebrates. Even in a lower vertebrate, zebrafish, complex-type *N*-glycans are among the major glycans expressed from an early embryogenetic period[3]. As a next step, alterations to *N*-glycan throughout embryogenesis or under various conditions should be examined. The expression pattern of *N*-glycans provided in this report would be useful for such a study.

REFERENCES

1. Chen, S., Spence, A.M., and Schachter, H. (2001) Complex *N*-glycans—when, why? *Trends Glycosci. Glycotechnol.* **13**, 447–462
2. Chen, S., Zhou, S., Sarkar, M., Spence, A.M., and Schachter, H. (1999) Expression of three *Caenorhabditis elegans N*-acetylglucosaminyltransferase I genes during development. *J. Biol. Chem.* **274**, 288–297
3. Schachter, H., Chen, S., Reinfold, V.N., and Spence, A.M. (2001) The role of complex *N*-glycans in metazoan development. *Glycoconj. J.* **18**, 44
4. Ioffe, E. and Stanley, P. (1994) Mice lacking *N*-acetylglucosaminyltransferase I activity die at mid-gestation, revealing an essential role for complex or hybrid *N*-linked carbohydrates. *Proc. Natl. Acad. Sci. USA* **91**, 728–732
5. Campbell, R.M., Metzler, M., Granovsky, M., Dennis, J.W., and Marth, J.D. (1995) Complex asparagine-linked oligosaccharides in *Mgat1*-null embryos. *Glycobiology* **5**, 535–543
6. Guerardel, Y., Balanzino, L., Maes, E., Leroy, Y., Coddeville, B., Oriol, R., and Strecker, G. (2001) The nematode *Caenorhabditis elegans* synthesizes unusual *O*-linked glycans: identification of glucose-substituted mucin-type *O*-glycans and short chondroitin-like oligosaccharides. *Biochem. J.* **357**, 167–182
7. Altmann, F., Fabini, G., Ahorn, H., and Wilson, I.B. (2001) Genetic model organisms in the study of *N*-glycans. *Biochimie* **83**, 703–712
8. Yanagida, K., Ogawa, H., Omichi, K., and Hase, S. (1998) Introduction of a new scale into reversed-phase high-performance liquid chromatography of pyridylamino sugar chains for structural assignment. *J. Chromatogr. A* **800**, 187–198
9. Lewis, J.A. and Fleming, J.T. (1995) Basic culture methods in *Methods in Cell Biology* (Epstein, H.F. and Shakes, D.C., eds.) Vol. 48, pp. 3–29, Academic Press, San Diego
10. Natsuka, S. and Hase, S. (1998) Analysis of *N*- and *O*-glycans by pyridylamination in *Methods in Molecular Biology* (Hounsell, E.F., ed.) Vol. 76, pp. 101–113, Humana Press, Totowa, NJ
11. Natsuka, S., Adachi, J., Kawaguchi, M., Ichikawa, A., and Ikura, K. (2002) Purification method of fluorescence-labeled oligosaccharides by pyridylamination. *Biosci. Biotechnol. Biochem.* **66**, 1174–1175
12. Makino, Y., Kuraya, N., Omichi, K., and Hase, S. (1996) Classification of sugar chains of glycoproteins by analyzing reducing end oligosaccharides obtained by partial acid hydrolysis. *Anal. Biochem.* **238**, 54–59
13. Suzuki, J., Kondo, A., Kato, I., Hase, S., and Ikenaka, T. (1991) Analysis by high-performance anion-exchange chromatography of component sugars as their fluorescent pyridylamino derivatives. *Agric. Biol. Chem.* **55**, 283–284
14. Kubelka, V., Altmann, F., Staudacher, E., Tretter, V., Marz, L., Hard, K., Kamerling, J.P., and Vliegenthart, J.F. (1993) Primary structures of the *N*-linked carbohydrate chains from honeybee venom phospholipase A2. *Eur. J. Biochem.* **213**, 1193–1204
15. Hase, S., Natsuka, S., Oku, H., and Ikenaka, T. (1987) Identification method for twelve oligomannose-type sugar chains thought to be processing intermediates of glycoproteins. *Anal. Biochem.* **167**, 321–326
16. Hase, S. and Ikenaka, T. (1990) Estimation of elution times on reverse-phase high-performance liquid chromatography of pyridylamino derivatives of sugar chains from glycoproteins. *Anal. Biochem.* **184**, 135–138
17. Ishimizu, T., Mitsukami, Y., Shinkawa, T., Natsuka, S., Hase, S., Miyagi, M., Sakiyama, F., and Norioka, S. (1999) Presence of asparagine-linked *N*-acetylglucosamine and chitobiose in *Pyrus pyrifolia* S-RNases associated with gametophytic self-incompatibility. *Eur. J. Biochem.* **263**, 624–634
18. Altmann, F., Schwihla, H., Staudacher, E., Glossl, J., and Marz, L. (1995) Insect cells contain an unusual, membrane-bound β-*N*-acetylglucosaminidase probably involved in the processing of protein *N*-glycans. *J. Biol. Chem.* **270**, 17344–17349
19. Watanabe, S., Kokuho, T., Takahashi, H., Takahashi, M., Kubota, T., and Inumaru, S. (2002) Sialylation of *N*-glycans on the recombinant proteins expressed by a baculovirus-insect cell system under β-*N*-acetylglucosaminidase inhibition. *J. Biol. Chem.* **277**, 5090–5093
20. van Tetering, A., Schiphorst, W.E., van den Eijnden, D.H., and van Die, I. (1999) Characterization of a core α1→3-fucosyltransferase from the snail *Lymnaea stagnalis* that is involved in the synthesis of complex-type *N*-glycans. *FEBS Lett.* **461**, 311–314