Presence of Fucosyl Residues on the Oligosaccharide Antennae of Membrane Glycopeptides of Human Neuroblastoma Cells

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ABSTRACT

Fucosyl residues linked α1→3 or 4 to N-acetylgalactosamine were found in large amounts on glycopeptides from the membranes of human tumor cells of neuroectodermal origin but not on membrane glycopeptides from human fibroblasts. The fucosyl residues were detected by release of radioactive fucose from the glycopeptides with an α-L-fucosidase specific for fucosylα1→3(4)-N-acetylgalactosamine. In other studies, the linkage was shown to be α1→3 by nuclear magnetic resonance analysis (U. V. Santer, M. C. Glick, H. van Halbeek, and J. F. G. Vliegenthart. Carbohydr. Res., 118: in press, 1983). Glycopeptides containing these fucosyl residues from four human neuroblastoma cell lines were defined by binding to immobilized lectins. In addition, the glycopeptides from one human neuroblastoma cell line, CHP-134, were further characterized by enzyme degradation and columns calibrated for size and charge. The anten- nary position of fucosylα1→3-N-acetylgalactosamine on the glycopeptides was demonstrated by the use of exoglycosidases and endoglycosidase D, since complete degradation to yield fucosyl-N-acetylgalactosaminylasparagine was obtained only after treatment with almon α-L-fucosidase prior to the sequential degradation.

Fucosylα1→3-N-acetylgalactosamine was present on most size and charge classes of membrane glycopeptides and therefore was not limited to a few glycoproteins. Since the almon α-L-fucosidase cleaves fucosyl residues from glycoproteins, the physiological effects of the increased specific fucosylation on human tumors of neuroectodermal origin can be examined.

INTRODUCTION

Human neuroblastoma cell lines have membrane glycopeptides similar to those of other tumor and virus-transformed cells (12, 15, 42). These cells also possess a variety of neuronal properties, including enzymes for neurotransmitter synthesis and excitable membranes (20). In addition, 2 human neuroblastoma cell lines, CHP-134 and MR-5, have been shown to contain a high percentage of the fucosyl residues as Fucα1→3(4)GlcNAc (12, 14, 32). In contrast, the membranes of mouse neuroblasto ma or other cell types examined, including human fibroblasts, did not contain significant amounts of these particular fucosyl residues (32). The fucosyl residues were detected with an α-L-fucosidase purified from almonds. This enzyme will cleave L-fucose-linked α1→3 or α1→4 to GlcNAc but not Fucα1→2Gal, Fucα1→6GlcNAc, or the usual synthetic fucosides (28, 46). Since the enzyme cannot distinguish Fucα1→3GlcNAc from Fucα1→4GlcNAc, high-resolution 1H NMR spectroscopy (40, 41) was used. In comparison with known oligosaccharides, these fucosyl residues in the neutral glycopeptides of CHP-134 cells were identified as Fucα1→3GlcNAc (33).

Fucα1→3GlcNAc has been shown previously to be present on the major glycan residue in rat brain (19) and in large amounts in mouse melanoma cells, changing when the properties of cell adhesion and metastasis changed (6, 7). Fucosyl residues in this linkage are found generally in only small amounts on certain serum glycopeptides (26, 40), although they have been reported to be a requirement for the uptake of human lactoferrin by mouse hepatocytes (30).

We report here the prevalence of Fucα1→3(4)GlcNAc on membrane glycoproteins of human cells of neuroectodermal origin and the characterization of the glycopeptides from one of the human neuroblastoma cell lines, CHP-134.

MATERIALS AND METHODS

Materials. L-[5,6,3H]Fucose, 56 Ci/mmol, or L-3H]fucose, 50.8 mCi/mmol, were obtained from New England Nuclear, Boston, Mass. DEAE-Sepacel, Sephacryl S-200, Con A-Sepharose, and lentil-Sephardase were from Pharmacia Fine Chemicals, Piscataway, N. J., and Immobilized Ulex I lectin was from E-Y Laboratories, San Mateo, Calif. Trypsin, 3 times crystallized, and soy bean trypsin inhibitor were from Worthington Biochemical Corp., Freehold, N. J. Vibrio cholerae neuraminidase was from Boehringer Mannheim Biochemicals, Indianapolis, Ind., Aerobacter ureafaciens neuraminidase was from E-Y Laboratories, and Pronase was from Calbiochem-Behring Corp., La Jolla, Calif. The starting material for the specific α-L-fucosidase from almonds was obtained as β-glucosidase* from Sigma Chemical Co., St. Louis, Mo. The purification and properties of a broad-spectrum α-L-fucosidase isolated from rat testes, β-o-galactosidase from baby rat ileum, and β-o-N-acetylhexosaminidase from porcine epididymis have been described (31). Endoglycosidase D and ManGlcNAc, Man3GlcNAc, and ManGlcNAc were from Dr. Michiko Fukuda, Cancer Research Foundation, La Jolla, Calif.; borotritide-reduced LNF I, LNF II, LNF III, disialyllacto-N-tetraose, and LS-tetrasaccharide c were from Drs. Victor Ginsburg and David Smith, NIH, Bethesda, Md. GlcNAcAsn was from Vega-Fox Biochemicals, Tucson, Ariz., and Man3GlcNAcAsn was prepared from ovalbumin as described (2).

Cell Culture and Characteristics. Human neuroblastoma cell (CHP-134) and human fibroblasts (CHP-134-F) from the patient containing tumor CHP-134 were grown as described (20, 34). SK-N-SH, NMB, IMR-32/5 and CHP-206 (Patient NB19), and CHP-212 (Patient NB9) were grown similarly and have been characterized by chromosomal analysis (9), as were human medulloblastoma cell line TE-761 (24) and retinoblastoma cell lines Weri (25), Y-79, and GM-1232 (8). The cells were made...
radioactive by growth in L-[^3H]- or L-[^14C]fucose (5 μCi/75-sq cm flask) added in fresh medium 48 hr before harvest. The cells were washed 5 times with 0.16 M NaCl, removed from the monolayer with trypsin (11), and pelleted. The supernatant material was further centrifuged at 27,000 × g and lyophilized and was the source of membrane glycopeptides.

The cell pellet was washed 3 times with 0.16 M NaCl, after which the cells maintained full viability, as measured by the exclusion of trypan blue. Neuroblastoma cells, CHP-134, had a protein content of 1 × 10^7 mg/cell and incorporated 1.8 × 10^6 cpm of [^3H]fucose per mg of protein. The fibroblasts, CHP-134-F, had a protein content of 3 × 10^7 mg/cell and incorporated 1.8 × 10^6 cpm per mg of protein. The trypsin-sensitive membrane material contained 10% of the total radioactive fucosylconjugates of the cell.

Preparation of Membrane Glycopeptides. The membrane glycopeptides obtained from the lyophilized material, which was removed from the cells by mild trypsin treatment, were digested exhaustively with Pronase (11) to obtain smaller glycopeptides. Free fucose and salts were removed by chromatography on a column of Bio-Gel P-2 (0.9 x 40 cm) in water. The major peak of the radioactivity was eluted in the first third of the column volume, when V₀ and Vₑ are measured with blue dextran as markers.

Preparation and Assay of α-L-Fucosidase. α-L-Fucosidase was isolated from almonds as described (28), with the exception that a second purification over Sephacryl S-200 was used. Borotritide-reduced milk oligosaccharides, LNF II and LNF III, were used as substrates. The enzyme did not hydrolyze LNF I. In order to follow the purification of α-L-fucosidase, a more rapid assay was developed. Glycopeptides of CHP-134 cells (5 μ containing 500 cpm) were incubated with the enzyme solution in 10 μl of Buffer A (0.2 M dibasic sodium phosphate and 0.1 M citric acid, pH 5.5) in a total volume of 25 μl for 16 hr. The released fucose was separated from the glycopeptides on a silica gel thin-layer chromatography plate after developing for 5 hr as described (1). All radioactivity remained at the origin in controls which were incubated with [^3H]fucose and developed as described (38) for monosaccharides. All radioactivity corresponded with that recovered by chromatography on Bio-Gel P-2. Radioactive fucose recovered by paper chromatography had a specific activity of 3750 cpm per nmol of fucose as measured by gas-liquid chromatography of the alditol acetate derivative (35).

Sequential Enzyme Degradation. Unless otherwise stated, for degradation of the glycopeptides, pooled [^3H]- and [^14C]-glycopeptides were suspended in 50 μl of Buffer A containing 1 mM CaC₂O₃, incubated at 37ºC for 4 hr with 1 unit of V. cholerae neuraminidase and 20 millinits of A. unaefaciens neuraminidase. After boiling, the mixture was incubated with 0.8 units of rat β-o-galactosidase, 2 units of bovine β-o-N-acetylhexosaminidase, and 100 μg of bovine serum albumin in a final volume of 100 μl and adjusted to pH 4.5 (31). After 48 hr, the 4 enzymes were added again at half-strength, and the incubation period was extended by 24 hr. Finally, the mixture was boiled, adjusted to pH 7.0, and incubated for 24 hr with 20 millinits of endoglycosidase D.

Chromatographic Procedures. In all cases, radioactivity was detected by the separation of the glycopeptides, pooled [^3H]- and [^14C]-glycopeptides were suspended in 50 μl of Buffer A containing 1 mM CaC₂O₃, incubated at 37ºC for 4 hr with 1 unit of V. cholerae neuraminidase and 20 millinits of A. unaefaciens neuraminidase. After boiling, the mixture was incubated with 0.8 units of rat β-o-galactosidase, 2 units of bovine β-o-N-acetylhexosaminidase, and 100 μg of bovine serum albumin in a final volume of 100 μl and adjusted to pH 4.5 (31). After 48 hr, the 4 enzymes were added again at half-strength, and the incubation period was extended by 24 hr. Finally, the mixture was boiled, adjusted to pH 7.0, and incubated for 24 hr with 20 millinits of endoglycosidase D.

Results

Distribution of the Almond Fucosidase-sensitive Linkage on Human Cells of Neuroradial Origin. It has been shown that 33% of the radioactive fucose was released by almond α-L-fucosidase from cellular or membrane radioactive material of human neuroblastoma cells, CHP-134, metabolically labeled with L-[^3H]fucose (32). Fucose was released by the α-L-fucosidase from the radioactive fucosylconjugates of the membranes of 5 other human neuroblastoma lines, 3 retinoblastoma lines, and 1 medulloblastoma cell line (Table 1). Although the amount of radioactivity released varied from 8 to 39% for the human neuroblastoma line TE-761.

<table>
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<th>Cell type</th>
<th>[^3H]Fucose released by α-L-fucosidase (Yo)</th>
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<td>33</td>
</tr>
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<td>CHP-134</td>
<td>18</td>
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<tr>
<td>IMR-32/5</td>
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<td>CHP-206</td>
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<tr>
<td>NMB</td>
<td>30</td>
</tr>
<tr>
<td>SK-N-SH</td>
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</tr>
<tr>
<td>Retinoblastoma</td>
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</tr>
<tr>
<td>Weri</td>
<td>30</td>
</tr>
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<td>Y-79</td>
<td>12</td>
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<td>3</td>
</tr>
<tr>
<td>TE-761</td>
<td>&lt;1</td>
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</table>

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neuroblastoma cell lines, it was more than that found on the fucoglycoconjugates from the membranes of 4 human fibroblast cell lines, including the fibroblasts, CHP-134-F, from neuroblastoma patient CHP-134. The 3 retinoblastoma cell lines examined had a similar proportion of radioactivity released by the enzyme. The medulloblastoma cell line, TE-761, had a small amount (3%) of almond enzyme-sensitive fucose. The latter was significant since, in addition to the human fibroblasts, there was never greater than 2% of these specifically linked fucosyl residues on many other cell types, including normal and transformed hamster fibroblasts and 4 mouse neuroblastoma cell lines, differentiated or nondifferentiated (32). The almond fucosidase is specific for greater than 2% of these specifically linked fucosyl residues on almond enzyme-sensitive fucose. The latter was significant.

The medulloblastoma cell line, TE-761, had a small amount (3%) of radioactivity released by the enzyme. The Pronase-digested material, which had a similar proportion of radioactivity, was eluted in the void volume. When Pronase-digested material was treated with almond fucosidase before rechromatography on Sephadex G-50, 33% of the radioactivity was released as free fucose (Chart 1B). Therefore, the fucosyl residues susceptible to almond fucosidase were on glycoproteins, having polypeptides degradable by Pronase, and not on glycolipids.

In order to determine whether or not the almond-susceptible linkage was present on a specific few or on many glycan residues, the membrane glycopeptides were collected according to size classes. The Pronase-digested membrane glycopeptides from CHP-134 cells were desalted on Bio-Gel P-2, separated on a column of Bio-Gel P-4, and combined into 4 groups (Chart 2A). When each of the radioactive fractions of membrane glycopeptide (Chart 2A) was treated with almond α-L-fucosidase and rechromatographed over Bio-Gel P-4 (Chart 2, A to E), each size class of glycopeptides showed the release of fucose (31, 24, 26, and 42%, respectively). Therefore, these specific fucosyl residues were present on glycan units of all sizes from many glycoproteins.

Position of Fucα1→3GlcNAc on Antennary Branches of Glycopeptides. Some of the fucosyl residues susceptible to almond fucosidase were on the antennary branches of complex-type glycopeptides with a mannosyl-chitobiose core. These glycopeptides also had fucosyl residues at the core. Chart 3 gives an example of a triantennary glycopeptide containing this structural configuration.

Endoglycosidase D, an enzyme with well-defined specificities (17), was used to demonstrate that the fucosyl residues were on antennary branches. The minimal substrate for this enzyme is Manα1→3Manβ1→4GlcNAcβ1→4GlcNAcX, where X may be α1→6Fuc and/or asparagine. The enzyme (Chart 3, Enzyme 5) cleaves the bond between the core GlcNAc residues (17). Thus, the antennary branches of glycopeptides must be removed to generate the substrate for endoglycosidase D. If the fucosyl residues were on an antennary branch, as shown in Chart 3, the substrate for endoglycosidase D could not be generated with the sequence of enzymes, neuraminidase (Enzyme 2), β-α-N-acetylhexosaminidase (Enzyme 3), and β-α-N-acetylhexosaminidase (Enzyme 4). If antennary fucose is first released by almond fucosidase (Enzyme 1), the other exoglycosidases can then produce an endoglycosidase D-susceptible core. The only radioactive products from fucose-labeled material after endoglycosidase D (Enzyme 5) action will be FucGlcNAc(Asn).

For these experiments, [14C]fucose-labeled glycopeptides of CHP-134 also were treated with almond α-L-fucosidase and mixed with 3H-glycopeptides which had been incubated without the almond enzyme. After chromatography on Bio-Gel P-2 to remove the released [14C]fucose, the remaining radioactive glycopeptides were treated with 4 enzymes: neuraminidase; β-galactosidase; β-N-acetylhexosaminidase; and endoglycosidase D. After this incubation period, the mixture was chromatographed on Bio-Gel P-4. Only 14C radioactivity was found in the region of FucGlcNAc and FucGlcNAcAsn (Chart 4A, Fractions 58 to 70). Thus, it was only after the fucosyl residues were removed from the antennary branches that any of the membrane glycopeptides were completely degraded by the series of enzymes.

The radioactive products of endoglycosidase D treatment (Chart 4A, Fractions 59 to 63 and 65 to 68) were characterized as charged and neutral molecules, respectively, by chromatography.
Fraction number

Chart 2. Size distribution of membrane glycopeptides containing the almond fucosidase-susceptible linkage. Membrane glycopeptides (4000 cpm) of CHP-134 eels, metabolically labeled with L-[3H]fucose, were Pronase digested and chromatographed on a column of Bio-Gel P-4 in 0.05 M ammonium acetate (A); aliquots were removed for determination of radioactivity. Fractions were combined as Groups 1 through 4, representing 20, 19, 47, and 14% of the total radioactivity, respectively, and were lyophilized. After incubation with 2 units of almond a-L-fucosidase for 72 hr, each group was rechromatographed on Bio-Gel P-4. B, Group 1; C, Group 2; D, Group 3; E, Group 4. Arrows positions in which blue dextran 2000 was eluted.

Chart 3. An example of a triantennary glycan linked to asparagine in the appropriate configuration for binding to immobilized lentil lectin. For a biantennary glycopeptide, the antennary branch containing GlcNAc1-4Man1-4 is not present, and this molecule will bind to either immobilized Con A or lentil lectin. The asterisk denotes that Fuc1-4 can be positioned on any or all of the antennary branches. The cleavage site is given in circled numbers for glycosidases: 1, almond a-L-fucosidase; 2, neuraminidase; 3, beta-galactosidase; 4, beta-glucosaminidase; 5, endoglycosidase D. Note that Enzyme 1 does not cleave Fuc1-6 at the core GlcNAc.

Chart 4. Radioactive profiles on Bio-Gel P-4 of the products of endoglycosidase D treatment. In A, [3H]fucose-labeled membrane glycopeptides from human neuroblastoma cells, CHP-134 (O), were incubated with 2 units of almond a-L-fucosidase for 72 hr, pooled with nontreated CHP-134 [3H]fucose-labeled membrane glycopeptides ( ), and chromatographed on Bio-Gel P-4. The higher-molecular-weight material (Fractions 27 to 43) was pooled and treated with a mixture of exoglycosidases and then with endoglycosidase D. The incubation mixture was rechromatographed on Bio-Gel P-4, and the radioactivity in each fraction was determined. In B, [3H]fucose-labeled membrane glycopeptides from human skin fibroblasts (CHP-134-F) were treated with almond fucosidase, a mixture of exoglycosidases, and then endoglycosidase D, and the mixture was chromatographed on Bio-Gel P-4. The column was calibrated with Man5GlcNAc2, Man4GlcNAc2, Man3GlcNAc2, Man2GlcNAc2, GlcNAc2, blue dextran (BD), and fucose (Fuc).
The characteristics of FucGlcNAc.

The charged product of complete enzymatic degradation (Chart 4A, Fractions 59 to 63) was retarded but not retained on Ulex I-agarose. The retardation was specific, since it was abolished by the inclusion of 0.2 M fucose in the eluting buffer. In addition, the glycopeptide migrated on paper (28), with \( R_{GlcNAcAsn} = 0.9 \), and was eluted from Dowex 50-H+ with a HCl gradient just before GlcNAcAsn. These characteristics and the Ulex I specificity (29) are the properties of FucGlcNAcAsn.

Presence of Fuc\( \alpha \)1->3GlcNAc on Glycopeptides Containing Sialic Acid. NeuAca2->6Gal and Fuc\( \alpha \)1->3GlcNAc have been shown to be mutually exclusive on an N-acetyllactosamine-containing antennary branch (29), although sialic acid and fucose have been shown to coexist on different antennary branches of the same glycan (44) or even on the same branch (19). Therefore, to determine whether or not the almond fucosidase-sensitive fucosyl residues and sialic acid were on the same glycopeptide, it was first determined that the almond fucosidase-susceptible linkage was present on all charge classes (Table 2).

Membrane glycopeptides from CHP-134 cells were separated on DEAE-Sepharose (Table 2, Part A). Although fucose was released by the almond fucosidase from each charge class, there was a decreasing percentage of the fucosidase-susceptible linkages with increasing charge.

In order to show whether or not the charge classes were attributable to sialic acid, the charged fractions were each pooled, treated with neuraminidase, and rechromatographed on DEAE-Sepharose. Seventy-seven % of the fucose-labeled molecules in these charged glycopeptides moved to positions of lesser charge, thus demonstrating that much of the charge was due to sialic acid (Table 2, Part B). Additional treatments with neuraminidase did not reduce the remaining charge.

The neuraminidase-treated fractions from DEAE-Sepharose were pooled as separate fractions, and each fraction was subsequently treated with almond fucosidase. Fucose was released from all of the fractions (Table 2, Part B), demonstrating the presence of fucose and sialic acid on the same glycopeptides.

The glycopeptides which had been separated by charge (Table 2) were each chromatographed on Bio-Gel P-4 before and after treatment with neuraminidase to determine the approximate size class. The desialylated glycopeptides from the most highly charged group were the largest, even after desialylation, and were comparable to those of Chart 2C. The neutral glycopeptides were the smallest and were comparable to those of Chart 2E.

Binding of Glycopeptides to Immobilized Lectins. The carbohydrate-binding specificities of immobilized lectins (5, 18) were used to show some additional structural characteristics of the glycopeptides of neuroblastoma cells, including the presence of fucosyl residues at the core. The presence of a fucosyl residue attached to the asparagine-linked N-acetylglycosamine and at least 2 \( \alpha \)-mannosyl residues are required for glycopeptide binding to the immobilized lentil lectin. Binding to lentil lectin (18) will occur if either or both of the \( \alpha \)-mannosyl residues of the glycopeptide are substituted at C-2, as in biantennary glycopeptides, or if one of the \( \alpha \)-mannosyl residues is substituted at C-2 and C-6, as illustrated in Chart 3. Other triantennary or tetraantennary glycopeptides will not bind. In contrast, binding to immobilized glycopeptides will not bind. In contrast, binding to immobilized glycopeptides will not bind. In contrast, binding to immobilized glycopeptides will not bind. In contrast, binding to immobilized glycopeptides will not bind. In contrast, binding to immobilized glycopeptides will not bind.

Con A is not dependent on fucosyl residues at the core, and the only complex-type glycopeptides bound are the biantennary type (2, 5).

The glycopeptides were applied to columns of Con A-Sepharose and lentil-Sepharose in series. The material which bound to each column was then separately eluted. Twenty-eight % of the membrane glycopeptides of CHP-134 bound to lentil lectin and not to Con A (Table 3). Only 12% of the glycopeptides bound to Con A-Sepharose. After treatment with fucosidase, an additional 3% of the glycopeptides bound to Con A.

The Con A nonbound, lentil lectin-bound membrane glycopeptides of CHP-134 cells were eluted in only 3 charge classes from DEAE-Sepharose (30, 50, and 300 nm buffer) as compared to the total membrane glycopeptides, which were found in 5 major classes (Table 2, Part A). The almond fucosidase-sensitive linkage was in the 3 charge classes (30, 30, and 15%, respectively) and was similar to the percentage found in the same classes of the total membrane glycopeptides (Table 2, Part A).

The membrane glycopeptides of 3 other neuroblastoma cell lines, SK-N-SH, NMB, and CHP-206, were examined for binding.

Table 2

<table>
<thead>
<tr>
<th>Column fraction</th>
<th>Elution buffer (mM)</th>
<th>% of total released by ( \alpha )-fucosidase</th>
<th>Radioactivity on Bio-Gel P-4 (V)</th>
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<tr>
<td>A. Original charge classes</td>
<td></td>
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<tr>
<td>A</td>
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<tr>
<td>B</td>
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<tr>
<td>C</td>
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<td>19</td>
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</tr>
<tr>
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<td>100</td>
<td>40</td>
<td>28</td>
</tr>
<tr>
<td>E</td>
<td>300</td>
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<td>18</td>
</tr>
<tr>
<td>B. After neuraminidase treatment and rechromatography</td>
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<tr>
<td>B &amp; B₁</td>
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<tr>
<td>D₃</td>
<td>100</td>
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</table>

* Calculated molarity at peak fraction.

4 U. V. Santer and M. C. Glick, unpublished results.
properties to immobilized Con A and lentil lectin (Table 3). As described for CHP-134, only a small percentage of fucose-containing glycopeptides bound to Con A. After removal of Fucα1→3GlcNAc, a larger percentage bound; however, at most, the proportion of the total membrane glycopeptides was 22%. The percentage of membrane glycopeptides binding to lentil lectin after Con A was 20 to 25% of the total glycopeptides. This amount did not change significantly after removal of Fucα1→3GlcNAc. Thus, 50% or more of the membrane glycopeptides of these human neuroblastoma cell lines did not have the proper configuration to bind to either immobilized Con A or lentil lectin.

Glycopeptides from Skin Fibroblasts of the CHP-134 Patient. CHP-134-F are skin fibroblasts from the patient with the neuroblastoma tumor CHP-134. Less than 1% of the incorporated radioactivity was released from membrane glycopeptides of CHP-134-F after treatment with almond fucosidase (Table 1).

In contrast to the neuroblastoma glycopeptides, 55% of the radioactivity of CHP-134-F membrane glycopeptides was retained by Con A-Sepharose (Table 3), indicating that more than one-half of the fucose-containing fibroblast membrane glycopeptides were of the biantennary glycan type. When these membrane glycopeptides were passed over Con A- and lentil-Sepharose in series, 18% bound to the lentil lectin.

The membrane glycopeptides from CHP-134-F were smaller than those of CHP-134 cells by chromatography on Bio-Gel P-4, since only 5% of the radioactivity was eluted in the void volume when compared with 20% for CHP-134 glycopeptides. Moreover, the desialylated membrane glycopeptides from CHP-134-F had an elution position on Bio-Gel P-4, V = 0.22 to 0.28, and lacked the larger glycopeptides (V = 0.16) found in the neuroblastoma membranes (Table 2, Part A).

More of the membrane glycopeptides of fibroblasts CHP-134-F were susceptible to endoglycosidase D (Chart 4B) than were those of CHP-134 cells (Chart 4A). Thus, when glycopeptides of CHP-134-F cells were treated with neuraminidase, β-galactosidase and β-N-acetylhexosaminidase, and endoglycosidase D (see Chart 3), the same profile of fucose-containing molecules of smaller size were generated as with CHP-134 after almond fucosidase pretreatment. However, twice the amount of radioactive fibroblast glycopeptides eluted as FucGlcNAcAsn or FucGlcNac, since more of the glycopeptides were degraded with the sequential enzymatic degradation.

**DISCUSSION**

The membrane glycopeptides of human neuroblastoma cells, CHP-134, contained unusually large amounts of Fucα1→3GlcNAc. These fucosyl residues were also demonstrated on other human cells of neureductodermal origin (Table 1). This was shown using an α-L-fucosidase from almonds specific for cleavage of Fucα1→3(4)GlcNAc. In other studies, which examined the neutral glycopeptides of CHP-134 cells, it was unequivocally shown by 500-MHz 1H NMR (40, 41) that the fucosyl residues were α1→3 and not α1→4 GlcNAc (33).

The glycopeptides, including those containing Fucα1-3GlcNAc, were partially characterized from membranes of 4 of the neuroblastoma cell lines by lectin affinity chromatography. The results showed that the binding affinities of the different cell lines were similar, since 10 to 20% bound to Con A and another 20% bound to lentil lectin after Con A (Table 3). Thus, the neuroblastoma cells contained only a small proportion of biantennary glycopeptides in the appropriate configuration to bind to Con A.

The membrane glycopeptides of one human neuroblastoma cell line, CHP-134, were characterized in greater detail. Although complete characterization will require NMR analysis (40, 41) of the individual glycopeptides, considerable information can be obtained from the methodology described here. Fucα1→3GlcNAc was shown to be on the antennary branch by degradation of the antennary glycan with neuraminidase, β-galactosidase, and β-N-acetylhexosaminidase (see Chart 3) and subsequent generation of FucGlcNAcAsn by endoglycosidase D (Chart 4). Only those glycopeptides which received prior treatment with α-L-fucosidase from almonds served as the endoglycosidase D substrate (13, 17) after degradation with exoglycosidases. The glycopeptides not treated were not degraded, since Fucα1→3GlcNAc on the antennary branch interfered with the action of β-galactosidase in addition to that of β-hexosaminidase (17). The change in charge class after neuraminidase treatment, followed by sizing, was consistent with sialic acid residues on 2 or more antennae (Table 2). The core structure which resulted from endoglycosidase D treatment (see Chart 3) was consistent with the binding specificity to immobilized Con A and lentil lectin. Most of the glycopeptides which were retained by lentil-Sepharose and not by Con A-Sepharose (Table 3) were presumably triantennary, with one α-mannose residue substituted at C-2 and C-6 (see Chart 3). This was the only class of glycopeptides from mouse lymphoma cells which showed these lectin affinities (18).

More than 50% of the glycopeptides did not bind to either lectin; therefore, the mannosyl residues may be substituted at C-2 and C-4, since these triantennary glycopeptides will not bind to lentil lectin. Alternatively, the glycopeptides may be tetra antennary; however, only a small portion of the Con A and lentil unbound glycopeptides bound to L-PHA. If the glycopeptides were triantennary and had one mannosyl residue substituted at C-2 and C-6, they would bind to L-PHA-agarose (5).
Although triantennary glycans were present, the amount cannot be calculated from these results, due in part to binding properties of certain biantennary glycopeptides to Con A (Table 3). Two Fuca1→3GlcNAc residues on a molecule that would otherwise be expected to be retained by Con A-Sepharose were reported to abolish binding, while only one residue decreased the affinity of binding (44). Therefore, the increase in binding of the fucose-labeled glycopeptides to Con A after treatment with α-fucosidase (Table 3) may be due to biantennary molecules with 2 fucosyl residues on the branches and one at the core. In addition, an intersecting GlcNAc (GlcNAcα1→4Manβ1→4GlcNAc... ) abolished the binding of some biantennary glycopeptides to Con A-Sepharose (2), although such compounds bind to lentil-Sepharose (43).

Fibroblasts from the patient with neuroblastoma tumor CHP-134 were available to rule out any glycopeptide changes due to variations of the individual. Consistent with the results from other fibroblasts (14), these fibroblasts had less than 1% of fucosyl residues as Fuca1→3GlcNAc on the membrane glycopeptides. Moreover, the major portion of the glycopeptides from the human fibroblasts bound to both Con A- and lentil-Sepharose, in contrast to those from the neuroblastoma cells (Table 3). This was in accord with the larger proportion of biantennary rather than triantennary glycopeptides reported for hamster fibroblasts when compared to virus-transformed cells (11, 31). In addition, similar to the hamster fibroblasts,4 proportionally more of the human fibroblast glycopeptides were susceptible to degradation with a series of exoglycosidases and endoglycosidase D than were those of the neuroblastoma cells (Chart 4).

It had been predicted on the basis of size, charge, and carbohydrate composition of purified glycopeptides from hamster cells that membrane glycoproteins of virus-transformed or tumor cells contain more highly branched oligosaccharide moieties than do their normal counterparts (10). The concept that these oligosaccharide changes impart particular characteristics to cells (12) has been reinforced by the consistency of this alteration among mammalian virus-transformed and tumor cells (12, 36, 42). Recently, using NIH-3T3 cells transformed by transfection with human DNA, a similar change was found (14); that is, a shift from a predominance of biantennary glycopeptides in the NIH-3T3 cells to tri- or tetrantennary glycopeptides in the transformed cells. The results presented here, contrasting 2 cell lines from the same patient, illustrate again the relative complexity of the tumor glycopeptides by 2 new criteria, sequential enzymatic degradation and sequential lectin chromatography. Moreover, the unusual presence of Fuca1→3GlcNAc on human tumors of neurectodermal origin may represent a specificity to a particular tumor.

Fuca1→3GlcNAc was found previously in blood group active oligosaccharides (21) and is part of the X-antigenic determinant of glycolipids (45) and SSEA-1, a putative stage-specific embryonic antigen (16). Most recently, it has been found on human adenocarcinoma of the colon or stomach (4), although only on glycolipids, in contrast to the neurectodermal tumor cells reported here, which contain the residues on glycoproteins.

Fuca1→3GlcNAc on an antenarrary branch influences the spatial configuration of carbohydride side chains and thus might be expected to impart biological specificity. No β-galactosidase is known which can release galactose from Galβ1→4(Fuca1→3GlcNAc→3)GlcNAc (17). Thus, urinary glycopeptides from fucosidosis patients which contain Fuca1→3GlcNAc also have a terminal galactose linked to the same GlcNAc (26). Furthermore, galactosyl residues in that configuration are no longer substrates for β-galactoside α2→6 sialyltransferase (3). The influence on structure is also shown by certain lectin specificities, as discussed (13). Fuca1→3GlcNAc on an antenarrary branch affected the binding of adjacent residues to lectins. It was shown (44) that some glycopeptides were not retained by Con A until the removal of these fucosyl residues, although the presence of this branch fucose did not inhibit the interaction between glycopeptides and lentil lectin when core fucose was present (Table 3). Moreover, in the neutral glycopeptide fraction, Fuca1→3GlcNAc inhibited binding to E-PHA-agarose (33). This lectin binds most glycopeptides containing intersecting GlcNAc (5).

In addition to neuroblastoma cells, other human tumors of neurectodermal origin, such as retinoblastoma, contained a larger proportion of fucosyl residues susceptible to almond α-L-fucosidase (Table 1) than did other human cell types examined. The fact that almond α-L-fucosidase was active on large glycopeptides and glycoproteins (32), in contrast to α-L-fucosidase from rat testes or other mammalian sources (31), means that it will be feasible to examine the physiological effects of the release of these specifically linked fucosyl residues.

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