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-acetylglucosamine 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 almond α-L-fucosidase specific for fucosylα1→3(4)-N-acetylglucosaminyl. 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 putative position of fucosylα1→3-N-acetylglucosaminyl on the glycopeptides was demonstrated by the use of exoglycosidases and endoglycosidase D, since complete degradation to yield fucosyl-N-acetylglucosaminylasparagine was obtained only after treatment with almond α-L-fucosidase prior to the sequential degradation.

Fucosylα1→3-N-acetylglucosaminyl was present on most size and charge classes of membrane glycopeptides and therefore was not limited to a few glycoproteins. Since the almond 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 excitatory membranes (20). In addition, 2 human neuroblastoma cell lines, CHP-134 and IMR-5, have been shown to contain a high percentage of the fucosyl residues as Fucα1→3(4)GlcNAc3 (12, 14, 32). In contrast, the membranes of mouse neuroblastoma 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 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 glycans 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. [5,6-3H]Fucose, 56 Ci/mmol, or L-[1-14C]fucose, 50.8 mCi/mmol, were obtained from New England Nuclear, Boston, Mass. DEAE-Sepharose, Sepharose 200, Con A-Sepharose, and lentil-Sepharose 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-acetythexosaminidase from porcine epididymis have been described (31). Endoglycosidase D and ManGalNACor, ManGalNACor, and ManGlcNAcor were from Dr. Michiko Fukuda, Cancer Research Foundation, La Jolla, Calif.; borotritide-reduced LNF I, LNF II, LNF III, disialylacto-N-tetraose, and LSTetrasaccharide c were from Drs. Victor Ginsburg and David Smith, NIH, Bethesda, Md. GlcNAcAsn was from Vega-Fox Biochemicals, Tucson, Ariz., and ManGalNACorAsn 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 CHP-134 were grown as described (20, 34). SK-N-SH, NMB, IMR-32/S 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 x 10^-7 mg/cell and incorporated 1.2 x 10^6 cpm of [3H]fucose per mg of protein. The fibroblasts, CHP-134-F, had a protein content of 3 x 10^-7 mg/cell and incorporated 1.8 x 10^6 cpm per mg of protein. The trypsin-sensitive membrane material contained 10% of the total radioactive fucoglyconjugates 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 V1 are measured with blue dextran 2000 and fucose.

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 dianium sulfate 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 without enzyme, whereas radioactivity co-migrated with fucose when the enzyme was present. With purified enzyme preparations, this method was linear with time and enzyme concentration. The pH dependence of the reaction was measured in phosphate-citrate buffer between pH 4.0 and 7.0, and the optimum of the enzyme activity, pH 5.5, in this assay matched that shown previously using LNF II as substrate (28). With this assay, fucose release was found to be maximal with 1.5 units of enzyme in 24 hr. These conditions were used for treatment of the membrane glycopeptides.

To verify the release of [3H]-monosaccharide as fucose, an incubation mixture (10,000 cpm) was applied to Whatman No. 1 cellulose paper and developed as described (38) for monosaccharides. All radioactivity which migrated away from the origin comigrated with fucose and 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 CaCl2, incubated at 37°C for 4 hr with 1 unit of V. cholerae neuraminidase and 20 million units of A. nesafacens neuraminidase. After boiling, the mixture was incubated with 0.8 units of rat b-galactosidase, 2 units of bovine b-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 million units of endoglycosidase D.

Chromatographic Procedures. In all cases, radioactivity was used to detect the separated material. Chromatography on a column of Sephadex G-50 in sodium dodecyl sulfate-Tris buffer (0.1 M Tris-acetate buffer, pH 9.0, containing 0.1% sodium dodecyl sulfate, 0.01% EDTA, and 0.1% mercaptoethanol) was as described (11). A column (0.9 x 80 cm) of BioGel P-4 was used for size separations of the glycopeptides and was eluted with 0.05 M ammonium acetate in 0.02% sodium azide. For charge separation of the glycopeptides, a column (0.9 x 16 cm) of DEAE-Sephacel was equilibrated in 0.5 M ammonium acetate, and the radioactive material was applied in the same buffer, followed by 20 ml of the buffer. A linear gradient from 0.5 to 50 M ammonium acetate (20 ml each) was followed by 15 ml of 50 M ammonium acetate, a second gradient from 50 to 100 M ammonium acetate (20 ml each) and then 15 ml of 100 M ammonium acetate, and a final gradient from 100 M to 1 M ammonium acetate (20 ml each). The [3H]-sorbitol derivative of monosialotetrasaccharide, LS-tetrasaccharide c (37) was eluted from this column with 60 M ammonium acetate, and disialylacto-N-tetraose was eluted with 100 M ammonium acetate. Other chromatographic markers were Man5GlcNAc2Asn and fucose, which were determined by the phenol sulfuric acid assay (38), and GlcNAcAsn. The latter was assayed by the Morgan-Elson procedure (38) after the release of GlcNAc following treatment with rat liver glycosylasparaginase, which was prepared as described (22). Man9GlcNAc2, Man9GlcNAc3, and Man9GlcNAc4 were detected by radioactivity.

Separation of glycopeptides on Con A-Sepharose (2, 27) and lentil-Sepharose (18) was performed as described. The eluted fractions were repurified on Bio-Gel P-2 before further enzyme treatment. Separation of glycopeptides on Ulex I-Agarose was carried out on a column (0.3 x 20 cm) by the method of Susz and Dawson (39). Separation of the glycopeptides on Dowex 50-H+ was done according to the method of Marshall and Neuberger (23).

**RESULTS**

**Distribution of the Almond Fucosidase-sensitive Linkage on Human Cells of Neurectodermal 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 glycoconjugates 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 cells.

<table>
<thead>
<tr>
<th>Table 1</th>
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<tbody>
<tr>
<td><strong>Distribution of membrane glycopeptides containing Fucα1→3 (4) GlcNAc</strong></td>
</tr>
<tr>
<td><strong>Cell type</strong></td>
</tr>
<tr>
<td>Neuroblastoma CHP-134</td>
</tr>
<tr>
<td>IMR-14</td>
</tr>
<tr>
<td>CHP-206</td>
</tr>
<tr>
<td>CHP-212</td>
</tr>
<tr>
<td>NMB</td>
</tr>
<tr>
<td>SK-N-SH</td>
</tr>
<tr>
<td>Retinoblastoma Weri</td>
</tr>
<tr>
<td>Y-79</td>
</tr>
<tr>
<td>GM-1232</td>
</tr>
<tr>
<td>Medulloblastoma TE-761</td>
</tr>
<tr>
<td>Fibroblasts CHP-134-F</td>
</tr>
</tbody>
</table>

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From the membranes of 4 human fibroblast 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 contained fucosyl residues in this uncommon nondifferentiated (32). The almond fucosidase is specific for fibroblasts and 4 mouse neuroblastoma cell lines, differentiated greater than 2% of these specifically linked fucosyl residues on the latter was significant because, 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, differentiates or nondifferentiates (32). The almond fucosidase is specific for Fucα1→3(4)GlcNAc; therefore, all of the cell lines of neurectodermal origin examined contained fucosyl residues in this uncommon linkage.

Presence of Fucα1→3GlcNAc on Glycopeptides of all Sizes. If radioactive fucose was released by the almond fucosidase from glycoproteins and not other glycoconjugates, the fucoglycoconjugates released with trypsin (see “Materials and Methods”) should be further degraded by a general protease. Therefore, the material from CHP-134 cells, which was eluted in the void volume of a Sephadex G-50 column (Chart 1A), was treated with Pronase and rechromatographed on Sephadex G-50 (Chart 1B). After Pronase digestion, none of the 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 poly-peptides 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 glycopeptides (Chart 2A) was treated with almond α-L-fucosidase and rechromatographed over Bio-Gel P-4 (Chart 2B), radioactive membrane glycoconjugates which were excluded from a Sephadex G-50 column equilibrated with water were pooled, lyophilized, treated with Pronase, boiled, and desalted. One portion (800 cpm) of the material was treated with 1 unit of almond fucosidase for 18 hr (○) and another (560 cpm) was incubated in buffer for the same time (□). Both were then successively chromatographed on Sephadex G-50 in sodium dodecyl sulfate-Tris buffer. BD and Fuc (bars), enzyme digestion position of blue dextran 2000 and fucose, respectively.

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→4GlcNAcb1→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 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-o-galactosidase (Enzyme 3), and β-o-N-acetylgalactosaminidase (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-acetylgalactosaminidase; 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 FucGlcNAc(Asn) (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 on Sephadex G-50. 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 on Sephadex G-50.

Fucosyl Residues on Neuroblastoma Glycopeptides

**Chart 1.** Glycoprotein nature of fucoglycoconjugates from CHP-134 cells. A, profile of the radioactive glycoconjugates (1100 cpm) removed by trypsin from the surface of CHP-134 cells, metabolically labeled with L-[14C]fucose, and chromatographed on a column of Sephadex G-50 in sodium dodecyl sulfate-Tris buffer (11). B, radioactive membrane glycoconjugates which were excluded from a Sephadex G-50 column equilibrated with water were pooled, lyophilized, treated with Pronase, boiled, and desalted. One portion (800 cpm) of the material was treated with 1 unit of almond fucosidase for 18 hr (○) and another (560 cpm) was incubated in buffer for the same time (□). Both were then successively chromatographied on Sephadex G-50 in sodium dodecyl sulfate-Tris buffer. BD and Fuc (bars), elution position of blue dextran 2000 and fucose, respectively.
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 α-L-fucosidase for 72 hr, each group was rechromatographed on Bio-Gel P-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—→6Man... is not present, and this molecule will bind to either immobilized Con A or lentil lectin. The asterisk denotes that Fucα1→3 can be positioned on any or all of the antennary branches. The cleavage sites are given in circled numbers for glycosidases: 1, almond α-L-fucosidase; 2, neuraminidase; 3, β-D-galactosidase; 4, β-D-glucosaminidase; 5, endoglycosidase D. Note that Enzyme 1 does not cleave Fucα1→6 at the core GlcNAc.

Chart 4. Radioactive profiles on Bio-Gel P-4 of the products of endoglycosidase D treatment. In A, [14C]fucose-labeled membrane glycopeptides from human neuroblastoma cells, CHP-134 (O), were incubated with 2 units of almond α-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 Man3GlcNAc2Asn, GlcNAcAsn, blue dextran (BD), and fucose (Fuc).
Fucosyl Residues on Neuroblastoma Glycopeptides

Table 2

Properties of membrane glycopeptides separated by charge

<table>
<thead>
<tr>
<th>Column fraction</th>
<th>Elution buffer (mM)$^a$</th>
<th>% of total</th>
<th>% released by α-L-fucosidase</th>
<th>Position on Bio-Gel P-4 (V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Original charge classes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>0.5</td>
<td>7</td>
<td>46</td>
<td>0.3</td>
</tr>
<tr>
<td>B</td>
<td>30</td>
<td>11</td>
<td>39</td>
<td>0.22</td>
</tr>
<tr>
<td>C</td>
<td>50</td>
<td>19</td>
<td>35</td>
<td>0.17</td>
</tr>
<tr>
<td>D</td>
<td>100</td>
<td>40</td>
<td>28</td>
<td>0.15</td>
</tr>
<tr>
<td>E</td>
<td>300</td>
<td>23</td>
<td>18</td>
<td>0.09</td>
</tr>
<tr>
<td>B. After neuraminidase treatment and rechromatography</td>
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</tr>
<tr>
<td>B' = B$_2$</td>
<td>0.5</td>
<td>6</td>
<td>42</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C' = C$_2$</td>
<td>0.5</td>
<td>16</td>
<td>35</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D' = D$_2$</td>
<td>0.5</td>
<td>20</td>
<td>33</td>
<td>0.22</td>
</tr>
<tr>
<td></td>
<td>50</td>
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<tr>
<td></td>
<td>100</td>
<td>6</td>
<td>13</td>
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</tr>
<tr>
<td>E' = E$_2$</td>
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<td>21</td>
<td>0.16</td>
</tr>
<tr>
<td></td>
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<tr>
<td></td>
<td>100</td>
<td>10</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>6</td>
<td>15</td>
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</tbody>
</table>

$^a$ Calculated molarity at peak fraction.

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 mM buffer) as compared to the total membrane glycopeptides, which were found in 5 major classes (Table 2, Part A). The aldehyde 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...
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Binding of membrane glycopeptides to immobilized lectins

Membrane glycopeptides were prepared as described in Table 1. An internal control of CHP-134 cell glycopeptides labeled with [14C]fucose was mixed with [3H]labeled glycopeptides from each of the other sources before chromatography and applied in 0.5 ml of buffer as described (18). Glycopeptides were chromatographed over Con A-Sepharose and lentil-Sepharose in series. The columns were then eluted with 0.2 M o-methyl mannoside. Free radioactive fucose was removed on Bio-Gel P-2 from those glycopeptides which were treated with almond fucosidase and a-L-fucosidase and /3-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 enzyme degradation.

DISCUSSION

The membrane glycopeptides of human neuroblastoma cells, CHP-134, contained unusually large amounts of Fuc1→3GlcNAc. These fucosyl residues were also demonstrated on other human cells of neurectodermal origin (Table 1). This was shown using an a-L-fucosidase from almonds specific for cleavage of Fuc1→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 a1→3 and not a1→4 GlcNAc (33).

The glycopeptides, including those containing Fuc1→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. Fuc1→3GlcNAc was shown to be on the antennary branch by degradation of the antennary branch with neuraminidase, /3-galactosidase, and /3-N-acetylhexosaminidase (see Chart 3) and subsequent generation of FucGlcNAcAsn by endoglycosidase D (Chart 4). Only those glycopeptides which received prior treatment with a-L-fucosidase from almonds served as the endoglycosidase D substrate (13, 17) after degradation with exoglycosidas.

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The glycopeptides not treated were not degraded, since Fuc1→3GlcNAc on the antennary branch interfered with the action of /3-galactosidase in addition to that of /3-hexosaminidase (17). The charge 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 tritennary, with one a-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 tritennary glycopeptides will not bind to lentil lectin. Alternatively, the glycopeptides may be tetramannosyl; however, only a small portion of the Con A and lentil unbound glycopeptides bound to L-PHA. If the glycopeptides were tetramannosyl and had one mannose residue substituted at C-2 and C-6, they would bind to L-PHA-agarose (5).

<table>
<thead>
<tr>
<th>Source of membrane glycopeptides</th>
<th>Treatment</th>
<th>Radioactivity bound (% of total)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Con A</td>
<td>Lentil after Con A</td>
</tr>
<tr>
<td>Neuroblastoma</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CHP-134</td>
<td>None</td>
<td>12 18</td>
</tr>
<tr>
<td></td>
<td>a-L-Fucosidase</td>
<td>15 27</td>
</tr>
<tr>
<td>CHP-206</td>
<td>None</td>
<td>12 25</td>
</tr>
<tr>
<td></td>
<td>a-L-Fucosidase</td>
<td>18 33</td>
</tr>
<tr>
<td>NMB</td>
<td>None</td>
<td>14 21</td>
</tr>
<tr>
<td></td>
<td>a-L-Fucosidase</td>
<td>17 20</td>
</tr>
<tr>
<td>SK-N-SH</td>
<td>None</td>
<td>22 21</td>
</tr>
<tr>
<td>Human fibroblasts</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CHP-134-F</td>
<td>None</td>
<td>55 18</td>
</tr>
</tbody>
</table>

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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 Fucα1→3GlcNAc residues on a molecule that would otherwise by 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 (GlcNAcβ1→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 Fucα1→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, 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 Fucα1→3GlcNAc on human tumors of neuroectodermal origin may represent a specificity to a particular tumor.

Fucα1→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 neuroectodermal tumor cells reported here, which contain the residues on glycoproteins.

Fucα1→3GlcNAc on an antennary branch influences the spatial configuration of carbohydrate side chains and thus might be expected to impart biological specificity. No β-galactosidase is known which can release galactose from Galβ1→4(Fucα1→3GlcNAc→3)GlcNAc (17). Thus, urinary glycopeptides from fucosidosis patients which contain Fucα1→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). Fucα1→3GlcNAc on an antennary 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, Fucα1→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 neuroectodermal origin, such as retinoblastoma, contained a larger proportion of fucosyl residues susceptible to aminoz α-L-fucosidase (Table 1) than did other human cell types examined. The fact that aminoz 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.

ACKNOWLEDGMENTS

We are indebted to Drs. Michiko Fukada and Victor Ginsburg for providing the materials, as described. The technical assistance of Florence Massey is acknowledged.

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

Ursula V. Santer and Mary Catherine Glick


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