Change in Glycosylation of Membrane Glycoproteins after Transfection of NIH 3T3 with Human Tumor DNA

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ABSTRACT

Altered glycosylation of membrane glycoproteins was demonstrated in NIH 3T3 cells transformed by transfection with DNA from human neuroblastoma and bladder carcinoma cell lines. The oncogenes of these two cell lines have been identified as N-ras and c-H-ras-1, respectively. The fucose-labeled membrane glycopeptides of transformation-induced transformants had decreased binding to concanavalin A-Sepharose when compared in dual-isotope experiments to those from NIH 3T3 cells, whereas binding to lentil lectin-Sepharose and leukoagglutinating phytohemagglutinin-agarose was increased. Binding affinities to these immobilized lectins lead to the interpretation of the results as a decrease in biantennary glycopeptides with a simultaneous increase in tri- or tetraantennary glycopeptides. Sephadex G-50 profiles also indicated a size increase of the glycopeptides of the transformants. None of these changes was growth related. This altered glycosylation, representing a heretofore unreported effect of the onc genes, may be necessary for the transformed phenotype.

INTRODUCTION

Membrane glycoproteins of mammalian cells contain bi-, tri-, and tetraantennary oligosaccharide residues. The expression of the more highly branched oligosaccharides on the glycoproteins of tumor cells appears to be part of the onogenic cascade (2, 14, 16, 38, 44). One element of the cascade has been identified by the transfer of transforming DNA sequences, the so-called oncogenes (6, 45), from human and animal tumor cell lines into the mouse cell line NIH 3T3 via transfection. Recently, there is evidence that at least 2 oncogenes may be necessary for the complete transformation of primary fibroblasts (24). Whether retroviruses or DNA translocations and amplification augment the expression of all of the cellular oncogenes and the products which are expressed is not currently clear (1, 11, 13). Nevertheless, transfection of NIH 3T3 provides a smaller and more manipulable portion of oncogenesis than was available previously and a system to examine a number of parameters usually associated with the oncogenic cascade.

Detailed studies using several transfected systems of NIH 3T3 cells have shown that the transforming sequence from human bladder carcinoma cells, T24, in NIH 3T3 is c-H-ras-1 (10, 21, 35), and a single base change leading to substitution of valine for glycine as the 12th amino acid has been reported in the gene product, p-21 (29, 39, 40). This mutation, however, may not be a general finding in solid tumors (43). On the other hand, the transforming sequence from human neuroblastoma cells, SK-N-SH, is only weakly homologous to v-H-ras and v-K-ras (36) and has thus been called N-ras (37). N-ras is located on chromosome 1, while other c-ras genes have been mapped to chromosomes 11, X, 6, and 12 (22). Thus, much has been learned about the DNA and protein sequences responsible for the transformation of NIH 3T3. The posttranslational changes, such as protein glycosylation, however, have not been as extensively investigated.

Affinity to immobilized lectins used in sequence should be useful in distinguishing glycopeptides from transformed cells from those of nontransformed cells, since the glycoproteins expressed on the transformed cells which have been examined in detail contain more highly branched oligosaccharide residues (16, 18, 26, 30). Lectin affinity characteristics and sequential separation of certain bi-, tri-, and tetraantennary glycopeptides have been described (7, 8, 23). Con A binds biantennary complex-type glycopeptides, as well as high-mannose glycopeptides (3). Certain triantennary glycopeptides bind to lentil lectin, i.e., those with one mannosyl residue substituted at C-2 and C-6, in addition to Fucα1→6 at the asparagine-linked GlcNAc (23). Lentil lectin (46) and EPHA (7) bind biantennary glycopeptides which have the sequence GlcNAcβ1→4Manβ1→4... called intersecting GlcNAc, but Con A does not (3). LPHA will bind galactosylated tri- and tetraantennary glycopeptides if one mannosyl residue is substituted at C-2 and C-6 (7). However, neither lentil nor LPHA will bind glycopeptides in which the α1→6 mannosyl residue is substituted at C-2 and C-4 rather than C-6. In addition to this fine specificity, there are a number of other factors which influence the binding affinities to specific lectins (3, 32, 33, 46, 47). Nevertheless, lectins used in sequence provide valuable tools for preliminary structural data which can be verified subsequently with high-resolution 1H-NMR (41, 42).

Using these immobilized lectins, we report altered glycosylation of the membrane glycoproteins of 2 different transformants generated by transfection of human DNA from cell lines with different oncogenes. This extends the range of effects of oncogenes to a major group of membrane components.

MATERIALS AND METHODS

Transfection with Human Neuroblastoma DNA. The method of transfection of NIH 3T3 was as described (27) with high-molecular-weight DNA from a human neuroblastoma cell line, SK-N-SH. Two and 3 weeks after transfection, foci were picked from monolayers. Using cloning rings, single cells were grown into colonies and tested for their ability to produce...
cells in semisolid agar and tumors in athymic "nude" mice. Cells (2 x 10^6) were injected s.c. into 2 sites/BALB/c-nu/nu mouse (Harlan/ Sprague-Dawley). In all cases, tumors were formed in less than 4 weeks with the transformants, T-1/3T3, but not with NIH 3T3, the recipient cells. A secondary transfection using T-1/3T3 DNA produced additional transformants, T-2/3T3. DNA from 3 different neuroblastoma cell lines was used to transfect NIH 3T3, but only DNA from SK-N-SH produced transformants.

Other Cell Types and Conditions of Culture. T24, a human bladder carcinoma cell line, was obtained from L. B. Chen, Sidney Farber Cancer Institute; Ki 3T3, Kirsten sarcoma virus-transformed NIH 3T3 cells, were obtained from C. D. Scher, Children's Hospital of Philadelphia. Cells a1-1 were obtained from M. Wigler, Cold Spring Harbor Laboratory, and were secondary transformants, that is, the result of a secondary transfection of NIH 3T3 by DNA from the original T24-transformed NIH 3T3 colonies in semisolid agar and tumors in athymic "nude" mice. Cells (2 x 10^6) were injected s.c. into 2 sites/BALB/c-nu/nu mouse (Harlan/ Sprague-Dawley). In all cases, tumors were formed in less than 4 weeks with the transformants, T-1/3T3, but not with NIH 3T3, the recipient cells. A secondary transfection using T-1/3T3 DNA produced additional transformants, T-2/3T3. DNA from 3 different neuroblastoma cell lines was used to transfect NIH 3T3, but only DNA from SK-N-SH produced transformants.

Preparation of Membrane Glycopeptides. For harvest, the cells were washed 3 times with Tris-buffered saline (20 mM Tris-HCl, pH 7.5, containing 0.15 M NaCl). Glycopeptides were removed from the fucose-labeled cells by controlled trypsinization (15). Trypsin (500 µg/75-sq cm flask) in 1 ml of Tris-buffered saline was added for 5 min at ambient temperature to the washed monolayer cells. The cells were removed by gentle shaking and were centrifuged at 300 x g for 5 min. The supernatant material was further centrifuged at 12,000 x g for 30 min and, after lyophilization, it was the source of membrane glycopeptides. The cells were further washed, and aliquots were removed for count, viability determination, and protein and radioactivity content. In all cases, the cells maintained more than 90% viability. The trypsin-releaseable membrane glycopeptides from cells grown in alternate isotypes were combined and further digested exhaustively with Pronase (15).

Separation of the Glycopeptides on Immobilized Lectins. Pronase-digested [14C]fucose and [3H]fucose-labeled glycopeptides were freed of low-molecular-weight material by chromatography on Bio-Gel P-2, suspended in 0.5 ml of Buffer A, and applied to a column (0.8 x 11 cm) of immobilized Con A-Sepharose (Pharmacia). The effluent from the column dropped directly onto a column (1.25 x 6 cm) of lentil lectin-Sepharose (Pharmacia), and the eluant from the lentil column was collected in 1-ml aliquots. The columns were washed with a total volume of 30 ml of Buffer A and then separated, and the bound glycopeptides were eluted from each column with 0.2 M D-methyl mannoside in Buffer A (8, 23).

The nonbound glycopeptides were desalted, lyophilized, resuspended in buffer as described (7), and chromatographed over a column (0.7 x 26 or 0.7 x 13 cm) of high-affinity LPHA-agarose (E. Y. Laboratories). The void volume was marked with cobalamin (33), and the bound glycopeptides were eluted by extensive washing (7). A similar procedure was used with a column (0.3 x 60 cm) of high-affinity EPHA-agarose (E. Y. Laboratories) which had been shown to reproducibly retain fucose-labeled glycopeptides from neuroblastoma CHP-134 (33). These glycopeptides thus were used as a standard.

The columns were regenerated with the appropriate buffers and stored in these buffers when not in use. The same columns were used for all determinations and maintained reproducible binding properties. The radioactive fractions were counted in a liquid scintillation counter, and the data were calculated and graphed by computer.

Filtration on Sephadex G-50. The [14C]fucose- or [3H]fucose-labeled fractions of trypsin-released material to be compared were lyophilized, combined, digested with Pronase, and chromatographed on a column (0.5 x 100 cm) of Sephadex G-50 (Pharmacia). Fractions (0.7 ml) were analyzed for radioactivity, and the data were calculated and graphed by computer (15).

Presence of Antennary FucO-1-3(4)GlcNAc. [3H]Fucose-labeled membrane glycopeptides were incubated with an α-fucosidase from almonds specific for fucosyl residues linked α1→3(4)GlcNAc (31). The release of fucose was quantitated with a [14C]fucose-labeled internal control after chromatography over Bio-Gel P-2 and by thin-layer chromatography as described (31).

RESULTS

Binding of Membrane Glycopeptides to immobilized Con A, Lentil Lectin, and LPHA. Table 1 gives the results of binding of the [3H]- and [14C]fucose-labeled membrane glycopeptides to immobilized Con A, lentil, and LPHA in series. When fucose-labeled membrane glycopeptides are examined under these conditions, Con A-Sepharose will remove biotannery glycopeptides, since fucosylated high-mannose glycopeptides have not yet been found (34); lentil lectin-Sepharose will then remove triantennary glycopeptides containing core fucose and mannose residues substituted at C-2 and C-6 and biantennary glycopeptides containing an intersecting GlcNAc (reviewed in Ref. 18); and LPHA-Sepharose will retain tetraantennary glycopeptides.

The percentage (41 and 38%, respectively) of fucose-labeled glycopeptides of both transformants, a1-1 and T-1/3T3, which bound to Con A-Sepharose was reduced when compared to that (65%) of NIH 3T3 (Table 1). In contrast, the percentage of a1-1

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Con A-bond</th>
<th>Lentil-bond</th>
<th>LPHA-bound</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transformant</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a1-1</td>
<td>41</td>
<td>35</td>
<td>16</td>
</tr>
<tr>
<td>T-1/3T3</td>
<td>38</td>
<td>42</td>
<td>14</td>
</tr>
<tr>
<td>Mouse recipient</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>NIH 3T3 (log)</td>
<td>58</td>
<td>20</td>
<td>ND</td>
</tr>
<tr>
<td>NIH 3T3 (confluent)</td>
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<td>17</td>
<td>7</td>
</tr>
<tr>
<td>Human donor</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T24 (bladder carcinoma)</td>
<td>37</td>
<td>9</td>
<td>34</td>
</tr>
<tr>
<td>SK-N-SH (neuroblastoma)</td>
<td>22</td>
<td>21</td>
<td>14</td>
</tr>
<tr>
<td>Virus-transformed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ki 3T3 (mouse)</td>
<td>25</td>
<td>26</td>
<td>40</td>
</tr>
<tr>
<td>Cb/Ba (hamster)</td>
<td>43</td>
<td>21</td>
<td>20</td>
</tr>
<tr>
<td>Nontransformed</td>
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<tr>
<td>BHk2/Cb (hamster)</td>
<td>63</td>
<td>12</td>
<td>16</td>
</tr>
</tbody>
</table>

*ND, not determined.

These glycopeptides were retained, but with lower affinity than those of the other cell lines which were examined (see Chart 1).
and T-1/3T3 glycopeptides (35 and 42%, respectively) which bound to lentil-Sepharose was increased when the transformants were compared to NIH 3T3. In the latter case, only 17% of the glycopeptides bound. As shown for NIH 3T3 (Table 1), the binding properties of the glycopeptides were not growth related.

Approximately 20% of the glycopeptides from the transformed cells, a1-1 and T-1/3T3, did not bind to either immobilized Con A or lentil lectin in series. Therefore, some of these unbound glycopeptides could be of the tetraantennary type which are retained by LPHA (7, 8). As shown in Table 1, the glycopeptides from the transformed cells, a1-1 and T-1/3T3, contained twice as many fucose-labeled glycopeptides which were retained by LPHA as those from the recipient, NIH 3T3 (14% as compared with 7%). The elution profile of Con A- and lentil-unbound glycopeptides of a1-1 and NIH 3T3 from a high-affinity, LPHA-agarose column is shown in Chart 1. The nonbound material from immobilized Con A, lentil, and LPHA-represented less than 10% of the total membrane glycopeptides from the transformants and NIH 3T3 cells. Thus, the 3 lectins in series accounted for the binding properties of the majority of fucose-labeled membrane glycopeptides from these mouse cell types.

It has been shown previously that antenary Fucα1→3GlcNAc changes the affinity of some glycopeptides for immobilized lectins (32, 33, 47). The transformants a1-1 and T-1/3T3 were examined for the presence of these linkages. Using almond α-L-fucosidase, no Fucα1→3(4)GlcNAc residues were detected under conditions which would have detected the presence of at least 2% of the fucosyl residues in these linkages.

Binding Properties of Human Tumor Glycopeptides to Immobilized Lectins. Human bladder carcinoma, T24, and human neuroblastoma, SK-N-SH, cell lines were the sources of DNA used to transfect NIH 3T3 and thus obtain a1-1 (40) and T-1/3T3. The surface glycopeptides from these human cell lines may reflect the tumor type, and it was therefore of interest to contrast the binding properties of their glycopeptides to the immobilized lectins in series with those of the mouse transformants.

The percentage of fucose-labeled membrane glycopeptides from T24 cells which bound to immobilized Con A was similar to those of its transformant (Table 1). However, a much higher percentage (34%) was retained by LPHA than lentil lectin (9%). The neuroblastoma cell line SK-N-SH had a low percentage of glycopeptides (22%) bound to Con A similar to that of another neuroblastoma investigated previously (31), and the binding to lentil lectin was higher than that of T24, so that approximately 40% of the glycopeptides were unbound to all 3 lectins and thus could not be accounted for by these procedures. About 33% of the fucosyl residues in SK-N-SH are antenary Fucα1→3GlcNAc (31), and these residues may contribute to the low lectin binding (33). No such fucosyl residues were found in T24 cells.

Lectin-binding Properties of Other Virus-transformed Cells. To test the general use of the lectin-binding affinities to distinguish glycopeptides of virus-transformed cells from their normal counterparts, the glycopeptides of Ki 3T3 cells were examined. Sixty-six % of the fucose-labeled glycopeptides from these Kirsten virus-transformed cells bound to either lentil or LPHA, and only a low percentage (25%) bound to Con A-Sepharose (Table 1). The majority of the fucose-labeled glycopeptides from fibroblasts of another species, hamster, bound to Con A-Sepharose, as did the mouse fibroblasts (NIH 3T3). The transformed counterpart showed a decrease in binding to Con A and increased binding to lentil lectin (Table 1).

Size Properties on Sephadex G-50. In order to show that the glycopeptides from the transformants a1-1 and T-1/3T3 were of higher molecular weight than those of the oncogene recipient, NIH 3T3, the fucose-labeled glycopeptides were chromatographed over Sephadex G-50. When the radioactive glycopeptides from a1-1 (Chart 2A) and T-1/3T3 (Chart 3A) were compared with those of NIH 3T3, a shift to higher molecular weight was observed in both transformants, and the profiles were the same. This shift was not growth related, and primary (T-1/3T3) and secondary (T-2/3T3) transformants showed the same profiles. This increase in size was greater and more heterogeneous for glycopeptides from virus-transformed cells, Ki 3T3 (Chart 2C), and human tumor cells T24 (Chart 2B) and SK-N-SH (Chart 3B).

To verify that the size distribution profile on Sephadex G-50 correlated with the lectin-binding properties of the glycopeptides, the Con A-, lentil-, and LPHA-bound glycopeptides were each chromatographed over Sephadex G-50. Dual-isotope experiments using membrane glycopeptides from a1-1 and NIH 3T3, as well as hamster cells, transformed and nontransformed (Table 1), showed that the glycopeptides bound to each lectin gave a symmetrical elution pattern. The profiles obtained showed a progression to larger size, with the largest glycopeptides binding to LPHA and the smallest binding to Con A. The profiles for a1-1 are shown in Chart 4.

Further Characterization of Lentil Lectin-bound Glycopeptides. The glycopeptides (Table 1) which bound to immobilized lentil lectin but not Con A have a fucosylated core and could be either triantennary with one mannosyl residue substituted at carbon atoms 2 and 6 (23) or biantennary with an intersecting GlcNAc (46). If also galactosylated, the former glycopeptides should be retarded by immobilized LPHA and the latter by EPHA (7). Thus, these immobilized lectins can be used for supporting...
Altered Glycosylation Accompanying Transfection

Chart 2. Profiles on Sephadex G-50 of fucose-labeled glycopeptides. [\(^{14}\text{C}\)]-Fucose-labeled glycopeptides from confluent a1-1 cells (•) were compared with [\(^{3}\text{H}\)]fucose-labeled glycopeptides (O) of NIH 3T3 (A), T24 bladder carcinoma cells (B), and Ki 3T3 (C). Blue dextran 2000 (BD) and phenol red, which was eluted after Fraction 70, served as markers. See "Materials and Methods." Information on the glycopeptide structures.

[\(^{14}\text{C}\)]Fucose- and [\(^{3}\text{H}\)]fucose-labeled glycopeptides from 2 cell types, which were Con A nonbound and lentil lectin bound, were mixed and applied to LPHA and EPHA, either separately (Table 2, Experiment 1) or in series (Table 2, Experiment 2). These experiments showed that, for the 4 cell lines examined, only a minor portion (12 to 25\%) of the lentil lectin-bound glycopeptides bound to EPHA and thus contained an intersecting GlcNAc. The major portion (60 to 78\%) bound to LPHA and, since these glycopeptides also bound to lentil lectin, they were probably of the triantennary type. As expected, none of the glycopeptides which bound to Con A bound to EPHA or LPHA.

DISCUSSION

A change in glycosylation of fucose-labeled membrane glycoproteins occurred after transfection of NIH 3T3 cells with DNA...
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Table 2
Comparison of lentil-bound glycopeptides from oncogene- and virus-transformed cells with normal counterparts

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Lentil-bound glycopeptides% of radioactivity</th>
<th>Total membrane glycopeptides; lentil-bound% of radioactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EPHA-bound</td>
<td>LPHA-bound</td>
</tr>
<tr>
<td>Experiment 1c</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a1-1</td>
<td>25</td>
<td>76</td>
</tr>
<tr>
<td>NIH 3T3</td>
<td>20</td>
<td>78</td>
</tr>
<tr>
<td>Experiment 2d</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ki 3T3</td>
<td>12</td>
<td>68</td>
</tr>
<tr>
<td>T-1/3T3</td>
<td>20</td>
<td>60</td>
</tr>
</tbody>
</table>

a Con A-nonbound, lentil-bound glycopeptides were eluted from lentil with 0.2 M a-methylmannoside in Buffer A and dialyzed and lyophilized.

b Calculated as percentages of the total membrane glycopeptides.

c Lentil-bound glycopeptides from a1-1 and NIH 3T3 labeled with L-[3H]fucose and [14C]mannose, respectively, were mixed, and aliquots were chromatographed on both EPHA and LPHA. The percentage retarded was calculated using cobaltam to measure the void volume (33).
d Lentil-bound glycopeptides from Ki 3T3 and T-1/3T3 labeled with L-[3H]fucose and [14C]mannose, respectively, were mixed and chromatographed on EPHA. Aliquots were taken, and the void volume, measured using cobaltamin and the peak of radioactivity, was collected, lyophilized, desalted, and chromatographed on LPHA.

from 2 different types of human tumors. The transforming genes from the 2 tumors have been isolated and are distinct. They are, however, both members of a single onc gene family, the ras genes, and the products of oncogene expression, 2-7 proteins, are cross-reactive immunologically (37). In both cases, the fucose-containing oligosaccharides from the membrane glycopeptides of the oncogene-transformed cells contained fewer biantennary glycopeptides. In other systems, such as endocytosis of glycopeptides by rat hepatocytes, the number of oligosaccharide branches influenced biological properties to a significant extent (4, 25), so it is proposed that a glycosylation change may be necessary for transfected cells to express the transformed phenotype.

The membrane glycopeptides of most, although not all (12, 17, 28), transformed and tumor cells thus far examined show a size increase as compared to those of the normal counterparts (2, 14, 16, 38, 44). It is interesting that, in most of the cases reported previously, transformation was accompanied by a size increase of the glycopeptides similar to that shown by Ki 3T3 or T24 cells (Chart 2). In contrast, the glycopeptides from the 2 oncogene-transformed cells (a1-1 and T-1/3T3) showed a less pronounced size increase on Sephadex G-50 (Charts 2 and 3). Since a1-1 and T-3T3 have the properties of transformed cells, it is possible that only a small percentage of glycopeptides needs to be altered for full expression of the transformed phenotype. The glycosylation change was more evident, however, as a reduction in the proportion of fucosylated glycopeptides which bound to Con A-Sepharose and an increase in those which bound to immobilized lentil lectin and/or LPHA (Table 1). Most of the glycopeptides which bound to lentil lectin also bound to LPHA (Table 2) and were presumably triantennary. Thus, transformation following the transfection of human tumor DNA into NIH 3T3 appears to result in a decrease in biantennary and an increase in tri- and tetraantennary fucose-labeled membrane glycopeptides. Further support is provided by the fact that the glycopeptides bound by immobilized Con A, lentil, and LPHA showed increasing size (Chart 4), as predicted by the binding affinities of known glycopeptides (7, 8, 23). Thus, the assignment of more highly branched oligosaccharides to the transfected cell glycoproteins is probably correct, even though not all tri- and tetraantennary glycopeptides from the other cell types could be detected by the lectins used here (Table 1). Since fucose-labeled glycopeptides were examined, it is possible, although contrary to what is known about the core fucosyl transferase (34), that altered fucosylation could account for the difference in Con A binding. NMR studies will have to be performed to determine the precise structures of these glycopeptides. High-resolution 500-MHz 1H-NMR can be used not only to analyze as little as 20 nmol of glycopeptide (41, 42) but also to analyze mixtures of glycopeptides (33). Therefore, it will be possible to obtain sufficient amounts of glycopeptides for further analysis from these cultured cells. However, it should be noted that these complete studies were performed on less than 10% of that amount.

While the glycopeptides from the 2 oncogene-transformed mouse cells showed a very similar pattern of binding to the lectins, the glycopeptides of the human cell lines which were the donors of the oncogenic DNA were distinctly different from the transformants. This was not a species difference, since a high percentage of the fucosylated surface glycopeptides from human fibroblasts were shown previously to bind to Con A-Sepharose (31). The lack of binding of a large portion of the membrane glycopeptides of human neuroblastoma tumor cells, SK-N-SH, to all 3 lectins (Table 1) was not surprising, since another human neuroblastoma cell line, CHP-134, gave a similar result (31, 33). It was postulated (31) that human neuroblastoma glycopeptides contained a mannose residue substituted at C-2 and C-4, since such a substitution interfered with binding (7, 23). Moreover, SK-N-SH glycopeptides contain 30% of the fucosyl residues as α1→3GlcNAc (19), and the presence of this fucose positioned on the oligosaccharide antennae influences binding. Indeed, Fucα1→3GlcNAc on the branch was shown to interfere with binding to Con A (47) and EPHA (33), although, conversely, it increased the binding of specific glycopeptides to lentil lectin (32). T24 does not contain Fucα1→3GlcNAc; thus, it is probable that the more highly branched glycopeptides shown by the elution profile of the Sephadex G-50 column (Chart 2B) have a predominance of mannosyl residues substituted at C-2 and C-4.

Our data suggest that, while the decrease in fucosylated biantennary glycopeptides in transformed cells is general, the type of branching in those glycopeptides which are concurrently increased may be species specific. In the transformed mouse cells, the oligosaccharide antennae are primarily of the type which can be detected with the lectins currently available. Long-term tumor lines of mouse (8) or virus-transformed cells like Ki 3T3 show a predominance of the same kind of branching. In contrast, in human and hamster cells, the larger glycopeptides may contain mannosyl residues substituted at C-2 and C-4 or have other configurations which prevent binding.

The absence of a specific N-acetylglucosaminyltransferase was described in an L-PHA-resistant mutant (9), and it was postulated that this enzyme was necessary to promote branching. It is possible that this enzyme is amplified during oncogenesis. However, overall glycosylation could change as the result of other factors affecting the oligosaccharide processing, such as the availability of nucleotide sugars or the rate of movement of proteins through the Golgi. Regardless of the mechanism, the same change in glycosylation is the consequence of the insertion of 2 different onc genes, albeit from the same ras family. Recently, myc or polyoma large T-antigen genes, along with the ras gene, have been shown to be necessary to transform primary
embryo fibroblasts to malignancy (24). Therefore, the ability to transform NIH 3T3, via the transfection procedure only after one oncogene is inserted, probably represents at least a second step of oncogenesis. It is at this step that we have demonstrated here an altered glycosylation. Further definition of this novel system should clarify the relationship of this oncogene-induced change and the transformation to malignancy.

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