Eur. J. Biochem. *181*, 249–260 (1989)
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N-linked oligosaccharide changes with oncogenic transformation require sialylation of multiantennae

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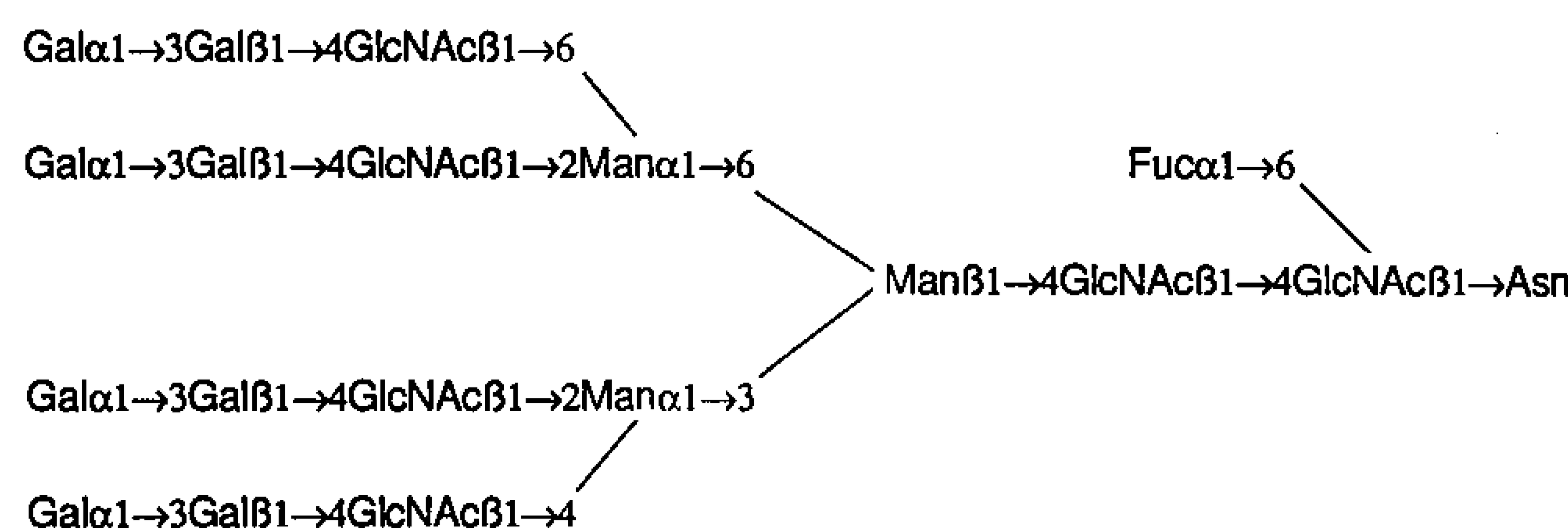
(Received July 12/November 17, 1988) – EJB 88 0844

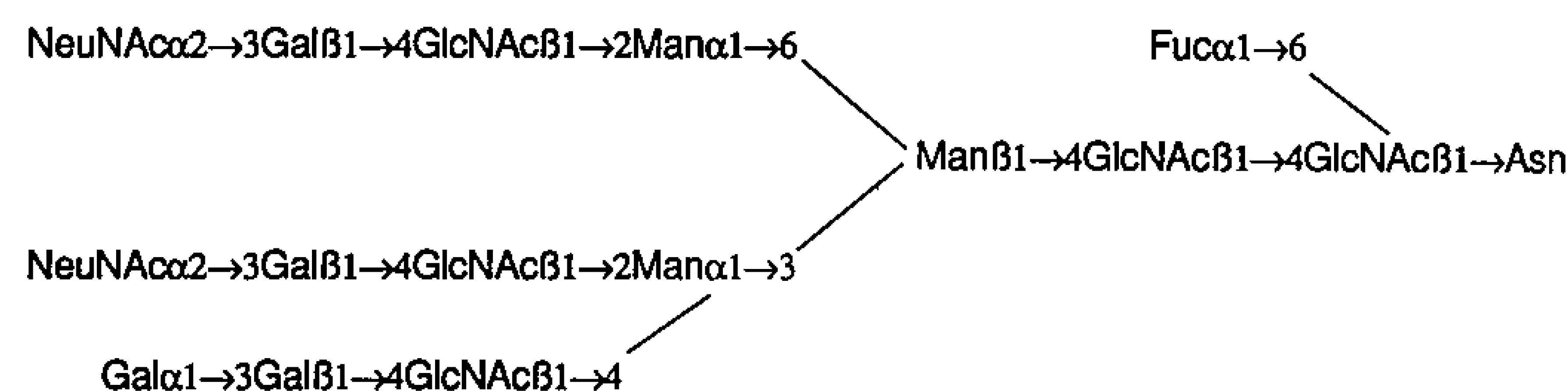
Glycopeptides derived from NIH 3T3 fibroblasts and these cells transformed by transfection with human DNA containing oncogene H-*ras* were analyzed by 500-MHz ^{1}H-NMR spectroscopy and binding to immobilized lectins. The cells were metabolically labeled with D-[^{3}H]glucosamine or L-[^{3}H]fucose and the glycopeptides included in Bio-Gel P-10 (M_r 5000–3500) were separated into neutral and charged fractions on DEAE-cellulose. The major portion (80%) of these [^{3}H]fucose glycopeptides from the non-transformed NIH 3T3 fibroblasts were neutral or contained one or two charged residues, whereas 90% of the glycopeptides from the transformed cells contained two or more charged residues.

The structure of the predominant neutral glycopeptide from the non-transformed NIH 3T3 cells was determined by ^{1}H-NMR spectroscopy to be tetraantennary containing terminal Galα1→3.



This structure was verified by binding to the immobilized α-Gal-specific lectin, *Griffonia simplicifolia* I and leukoagglutinating phytohemagglutinin from *Phaseolus vulgaris* (L-PHA), which binds certain tri- or tetraantennary glycopeptides.

In contrast, the structure derived by NMR spectroscopy of one of the predominant charged glycopeptides from the transformed cells was triantennary containing terminal NeuNAcα2→3 in addition to Galα1→3.



In attempting to verify this structure by lectin-binding properties it was found that removal of NeuNAcα2→3 reduced the affinity to L-PHA–agarose. The other major glycopeptides of the transformed cells which were more charged also cotained NeuNAcα2→3 but no NeuNAcα2→6 or Galα1→3.

A tentative structure was proposed for the major glycopeptide of the first charged class from NIH 3T3 cells on the basis of lectin-binding properties and the NMR spectrum which showed, in addition to NeuNAcα2→3, the presence of NeuNAcα2→6 and Galα1→3.

On the basis of the NMR spectrum and other results, it is concluded that the presence of tetraantennary oligosaccharides are not sufficient for the transformed oligosaccharide phenotype. Rather, the tri- or tetraantennae must be sialylated in α2→3 linkage, on more than one antennae, when properties of transformation are expressed in NIH 3T3 cells. Prior to transformation the tetraantennary oligosaccharides of these cells are terminated in α-Gal residues, whereas after transformation α-Gal residues appear to be replaced by NeuNAcα2→3. Thus, in this case, transformation may be accompanied by a change in the glycosyltransferases responsible for termination of the branches rather than those conferring branch configurations.

Glycoproteins of transformed and tumor cells contain oligosaccharides of increased size when compared with non-transformed cells (see reviews [1–3]). A greater degree of sialylation and the multiantennary structure of the oligosaccharides were subsequently shown to be responsible for the size increase. These conclusions were based on the compositional analysis of membrane glycopeptides [4] and structural characteristics of one of the major glycopeptides [5] from transformed hamster cells. Other laboratories have confirmed these findings [6, 7] and extended them to propose that the enzyme, GlcNAc transferase V, which confers the second antenna to the Manα1→6 branch of the oligosaccharide is responsible for the glycosylation alteration accompanying transformation [8].

A glycosylation inhibitor, swainsonine, which prevents full processing of the Manα1→6 antenna of the oligosaccharides [9] also prevents expression of a transformed phenotype, growth in soft agar, of transformed NIH 3T3 cells [10] and the metastatic ability of mouse melanoma cells [11]. Both of these observations were the first direct proof of the necessity of appropriately glycosylated glycoproteins for tumor-related properties and support a role for the Manα1→6 antennae in the multiantennary oligosaccharide hypothesis of transformation.

We now show that the multiantennary oligosaccharides may be necessary but are not sufficient for complete expression of the transformed phenotype. Using NIH 3T3 fibroblasts and these cells transformed by oncogene, H-*ras* [12, 13], we found that a large percentage of oligosaccharides were multiantennary prior to transformation and were terminated with α-Gal residues. After transformation most of the β-Gal residues were substituted by NeuNAc residues in α2→3 linkage and not by α-Gal residues.

EXPERIMENTAL PROCEDURES

Cell lines, culture and harvest

Mouse fibroblasts, NIH 3T3 and these cells transformed, a1-1, by transfection with DNA from human bladder carcinoma cell line, T-24, containing oncogene H-*ras* [12, 13], were obtained from Dr Michael Wigler, Cold Spring Harbor Laboratories. The cells were grown and harvested as described [12] and were used between the second and fourth passages. Each flask (75 cm^2) was labeled for 48 h prior to harvest with 5 μCi L-[5,6-^{3}H]fucose (60 Ci/mmol) or D-[6-^{3}H(n)]glucosamine (31 Ci/mmol, n = nominal labeling position) obtained from NEN. In designated experiments, the cells were treated with 5 μg swainsonine (purchased from Dr E. A. Dorling, Murdock University, Australia) per ml medium for 24 h in the presence of either isotope, and a similar procedure was used for the untreated cells [10]. All tissue culture medium and fetal calf serum were from Hazelton Research Products, Inc. (Denver, PA).

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Abbreviations. Fuc, L-fucose; α-MeGlc, methyl α-D-glucoside; Con A, concanavalin A; GSI, lectin I from *Griffonia simplicifolia*; L-PHA, leukoagglutinating phytohemagglutinin from *Phaseolus vulgaris*; RCAI, lectin I from *Ricinus communis*; SNA, lectin from the bark of *Sambucus nigra* L.

Partial purification of glycopeptides

The washed NIH 3T3 and a1-1 cells (3.2×10^9 and 1.3×10^9, respectively) which remained viable after harvest, were exhaustively digested with pronase [14] and the digested material was filtered through glass wool. The [^{3}H]glycopeptides were separated on a column (2.8 × 28 cm) of Bio-Gel P-10 in 50 mM ammonium acetate. The included material, M_r 5000–1350, was rechromatographed on a column (1 × 90 cm) of Bio-Gel P-10 in the same buffer. Charge separation was by chromatography on a column (1.3 × 22 cm) of DEAE-cellulose, equilibrated with 0.5 mM sodium phosphate buffer, pH 6.8, then eluted with a linear gradient to 30 mM sodium phosphate, pH 6.8, and finally with 100 mM sodium phosphate, pH 6.8, as described [14]. The fractions were combined according to the radioactivity profile and desalted by Bio-Gel P-2, twice, for NMR analysis. All other desalting was by Bio-Gel P-2. In some cases, an analytical column (0.9 × 12 cm) of DEAE-cellulose was used.

Lectin chromatography

Immobilized ConA–Sepharose and lentil–Sepharose (Pharmacia) and L-PHA–agarose (E. Y. Laboratories) were used as described [12] following the methods of Cummings and Kornfeld [15] and Kornfeld et al. [16]. The fractions which were eluted from L-PHA–agarose with 2.5 column volumes of buffer were designated 'bound' rather than retained as previously described [15] and those which were eluted prior to this but after the void volume were designated 'retarded'. *Griffonia simplicifolia* I lectin, GSI–agarose (Vector) was eluted with four column volumes of buffer at 4°C and then with 10 mM α-MeGal in the same buffer in order to distinguish the presence of one or more α-Gal residues according to Spiro and Bhoyroo [17]. [^{14}C]UDP-Gal (NEN) was included with the unknown fraction since it was retarted on this column [18]. RCAI–agarose (E. Y. Laboratories) was used as described [19]. SNA (E. Y. Laboratories) was coupled to activated Sepharose (Pharmacia) and the final product contained 1.2 mg lectin/ml gel. The column (0.8 × 4 cm) was eluted as described [20]. Serotonin-Sepharose was prepared and used as described [21]. In all cases the columns were standardized with radioactive glycopeptides of known binding characteristics [12, 22–24].

Enzyme treatment

Vibrio cholerae neuraminidase (0.02 U; Calbiochem) was incubated with the designated glycopeptide in 0.1 M citrate phosphate buffer, pH 5.5, containing 1 mM $CaCl_2$ for 15 h at 37°C. Coffee bean α-galactosidase was from Sigma. The enzyme (0.08 U) was incubated with the glycopeptides in 0.15 M acetate buffer, pH 5.0, for 15 h at 37°C [25].

High-resolution ^{1}H-NMR spectroscopy

A Bruker AM-500 spectrometer (SON hf. NMR facility, Department of Biophysical Chemistry, Nijmegen University and Department of Organic Chemistry, Utrecht University, The Netherlands), operating in the Fourier-transform mode, and equipped with an Aspect-3000 computer, was used. Further experimental details were as described previously [26, 27]. Prior to analysis, the glycopeptides were treated repeatedly with deuterium oxide at room temperature, with intermediate lyophilization. Finally, the samples were redissolved

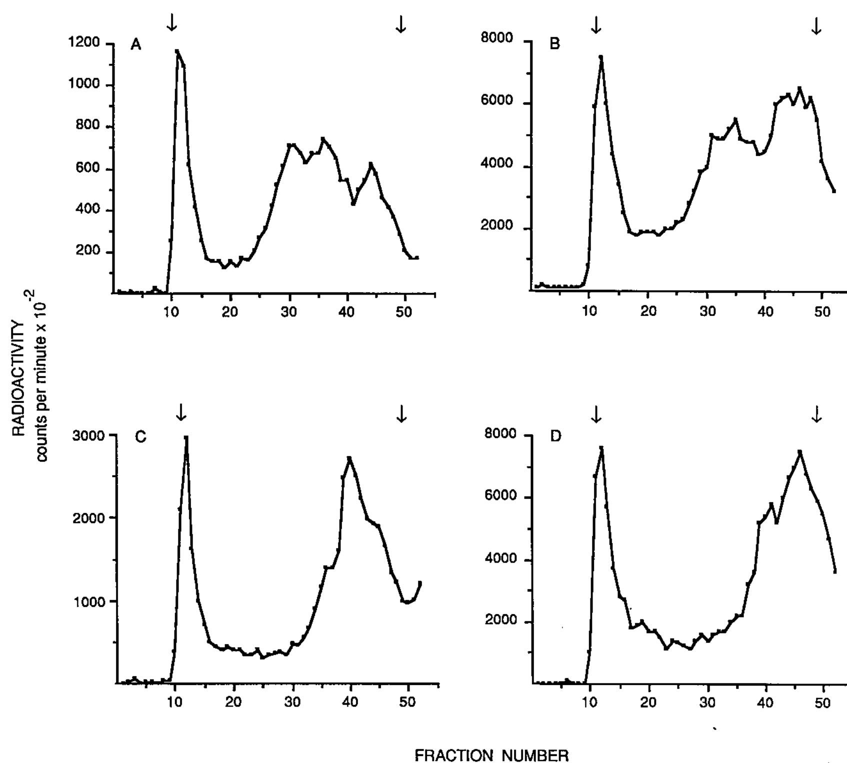


Fig. 1. *Profile on Bio-Gel P-10 of glycopeptides from (A, C) a1-1 and (B, D) NIH 3T3 cells, (A, B) untreated and (C, D) swainsonine-treated.* (A) a1-1 and (B) NIH 3T3 cells were labeled metabolically with D-[^{3}H]GlcN, harvested, digested with pronase and chromatographed on Bio-Gel P-10. (C) a1-1 and (D) NIH 3T3 cells were treated for 24 h with swainsonine (5 µg/ml) prior to harvest [10]. For (A) a1-1 cells, fraction B = fractions 25–40; for (B) NIH 3T3 cells, fraction B = fractions 22–40. Arrows represent (left) the position of the void volume of the column and (right) cobalamin, M_r 1350

in 0.4 ml ^2H_2O (99.96 atom % ^{2}H, Aldrich). Resolution enhancement of the spectra was achieved by Lorentzian-to-Gaussian transformation [26, 27]. The probe temperature was 300 K. The chemical shifts (δ) are expressed in ppm downfield from the methyl signal of internal sodium 4,4-dimethyl-4-silapentane-1-sulfonate, but were actually measured by reference to internal acetone (δ = 2.225 ppm) with an accuracy of 0.002 ppm.

RESULTS

Separation of glycopeptides and rationale for selection of specific glycopeptides

The pronase-digested glycopeptides from NIH 3T3 and the oncogene-transformed cells, a1-1, were separated on Bio-Gel P-10 into several size classes. The typical profiles of [^{3}H]GlcN-glycopeptides show that the transformed cells (Fig. 1A) had proportionately more material which was eluted with an apparent larger size compared with that of NIH 3T3 (fractions 25–40, Fig. 1A and B). NIH 3T3 (Fig. 1B) showed proportionately more radioactive material of smaller size (fractions 40–50, Fig. 1A and B). The glycopeptides from fractions 25–40 (Fig. 1A and B) M_r 5000–3500 (fraction B) were combined for each cell type, lyophilized, desalted and further separated on DEAE-cellulose as shown in Fig. 2A and B. As apparent from the profile, the glycopeptides from a1-1 cells (Fig. 2B) were more highly charged than those of NIH 3T3 (Fig. 2A). This is summarized in Table 1 where the percentage distribution is given for both metabolic labels, [^{3}H]Fuc and [^{3}H]GlcN. Note however, that the first charged

Table 1. *Characteristics of glycopeptide fraction B*
The glycopeptides M_r 5000–3500, which were eluted from Bio-Gel P-10 (Fig. 1A and B) and called fraction B, were derived from the total radioactive glycopeptides M_r 5000–1500. The charged properties of fraction B were defined by the profile on DEAE-cellulose (Fig. 2). Glycopeptides which did not bind when eluted with 0.5 mM sodium phosphate buffer, pH 6.5, were classed as neutral. The charged glycopeptides were classed according to the elution pattern with increasing concentration of buffer [14]. Therefore glycopeptides which were eluted with up to 16 mM sodium phosphate buffer, pH 6.5, were classed as containing one or two (1–2) NeuNAc residues and all the [^{3}H]glycopeptides which were eluted thereafter were classed as containing two or more NeuNAc residues (>2 NeuNAc)

	Radioactivity in			
	NIH 3T3		a1-1	
Glycopeptide	[^{3}H]GlcN	[^{3}H]Fuc	[^{3}H]GlcN	[^{3}H]Fuc
	% of glycopeptides M_r 5000–1500			
Total	32	42	50	52
	% of fraction B			
Neutral	17	24	3	7
Charged				
1–2 NeuNAc	49	56	34	47
>2 NeuNAc	31	19	62	46

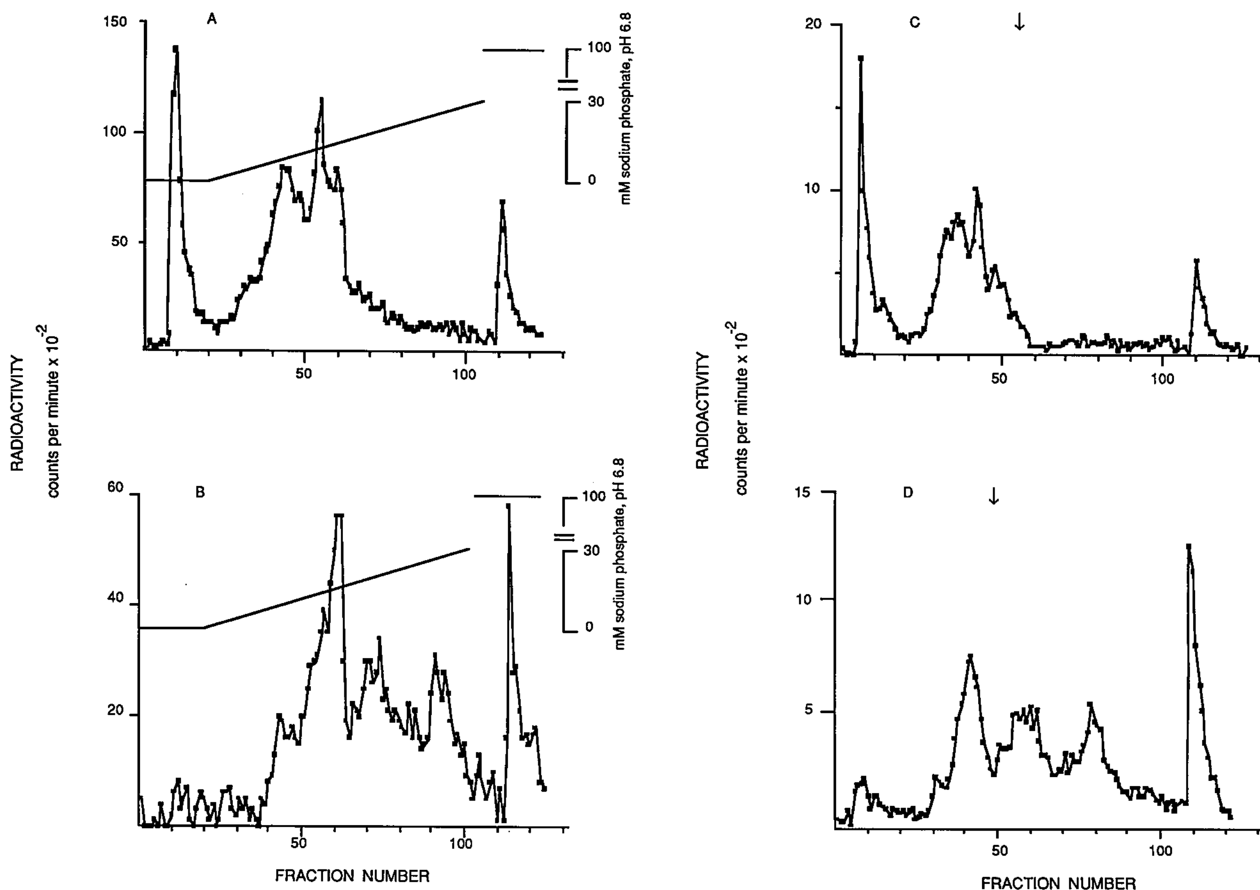


Fig. 2. *Chromatography on DEAE-cellulose of fraction B from (A, C) NIH 3T3 and (B, D) al-1 cells.* Fraction B (see Fig. 1) was from cells labeled metabolically with (A, B) D-[^{3}H]GlcN or (C, D) L-[^{3}H]Fuc. The fractions were separated for each cell type according to the profile. Fractions (A) 6–28, (B) 6–29 were designated neutral glycopeptides; fractions (A) 29–51 and 52–62, (B) 30–62 were designated charged glycopeptides containing 1–2 NeuNAc residues; fractions (A) 63–104 and 109–125, (B) 63–85, 86–104 and 110–125 were designated charged glycopeptides containing two or more NeuNAc residues. (C, D) The arrow designates the gradient at 16 mM phosphate buffer, the separation point of 1–2 NeuNAc residues from two or more NeuNAc residues

group of [^{3}H]GlcN-labeled glycopeptides of NIH 3T3 cells (Fig. 2A) was eluted from DEAE-cellulose prior to that of the a1-1 cells (Fig. 2B; 9.5 mM and 15 mM phosphate buffer, pH 6.8, respectively). These charged glycopeptides from NIH 3T3 cells were positioned as one charged (NeuNAc) residue whereas those of the a1-1 cells could have two charged (NeuNAc) residues.

With both cell types, fraction B was 40–50% of the total fucosylated glycopeptides which were included in Bio-Gel P-10. In fact, fraction B could comprise more than 75% of the cell-derived glycopeptides since much of the [^{3}H]GlcN material which was not included in Bio-Gel P-10 (Fig. 1) may be proteoglycans and the material smaller than fraction B may be incompletely processed glycopeptides or degradation products as seen in other systems [14, 28]. Thus fraction B is representative of the major group of glycopeptides from both cell types.

NEUTRAL GLYCOPEPTIDES

NMR characterization of glycopeptides from NIH 3T3 cells

The neutral glycopeptides of NIH 3T3 which were not bound to DEAE-cellulose (Fig. 2A), comprised 25% of [^{3}H]Fuc-labeled fraction B, and were analyzed by 500-MHz ^{1}H-NMR spectroscopy. Table 2 gives the structural features of the major component derived from the chemical shifts of structural reporter groups and those of two reference compounds. The surprising feature was the presence of a tetraantennary glycopeptide which contained terminal α-Gal residues. The *N*-acetyl signals, as well as the H-1 and H-2 signals of Man, suggest a tetraantennary oligosaccharide. Terminal α-Gal residues were inferred from the Gal H-1 signal at 5.145 ppm and Gal H-5 signal at 4.192 ppm. The 1→3-type of linkage was substantiated by the resonance position of H-4 of Galβ1→4 at 4.181 ppm. The intensity of these signals suggested that all four branches are terminated with an α-Gal residue. Fucα1→6 linked to GlcNAc-**1** is characterized by the structural-reporter groups of Fuc (H-1 and CH_3 see Table 2). Previously, a tetraantennary structure with three terminal Galα1→3 residues and one NeuNAcα2→6 residue has been published [29]. Thus, the NMR data are in agreement with the tentative structure for the oligosaccharide of the predominant glycopeptide as given in Fig. 3A.

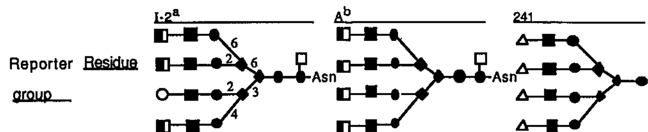
Lectin affinity characterization of glycopeptides from NIH 3T3 cells

Verification that the predominant neutral glycopeptide was tetraantennary with terminal α-Gal residues came from

Table 2. [1]*H-Chemical shifts of structural-reporter group protons of the constituent monosaccharides of the main component (A) of the neutral glycopeptides of NIH 3T3 cells*
The neutral glycopeptides are compared with those for the reference compounds I-2 [29] and 241 (van Pelt and Vliegenthart, unpublished results). Chemical shifts are given at 500-MHz for neutral solutions in D_2O at 300 K. Values are in ppm downfield from sodium 4,4-dimethyl-4-silapentane-1-sulfonate (DSS) using internal acetone at 2.225 ppm. Compounds are represented by short-hand symbolic notation [27]. For numbering of the monosaccharide residues see Figure 3.

●, GlcNAc; ■, β-Gal; ◧, α-Gal; ◆, Man; △, NeuNAcα2→3; ○, NeuNAcα2→6; □, Fucα1→6; n.d., not determined

Compound and schematic structure



Reporter group	Residue	I-2[a]	A[b]	241
H-1	GlcNAc-1	5.075	5.058	
	GlcNAc-2	4.681	4.678	5.204
	Man-3	4.77	n.d.	n.d.
	Man-4	5.14	5.121	5.132
	Man-4'	4.882	4.874	4.864
	GlcNAc-5	4.601	4.553	4.561
	GlcNAc-5'	4.59	4.553	4.580
	Gal-6	4.443	4.535	4.545
	Gal-6'	4.540	4.535	4.545
	GlcNAc-7	4.556	4.594	4.545
	GlcNAc-7'	4.556	4.594	4.545
	Gal-8	4.540	4.535	4.545
	Gal-8'	4.540	4.535	4.561
	Galα1→3	5.148	5.145	-
	Fucα1→6	4.88	4.874	-
H-2	Man-3	4.208	4.206	α4.219,β4.202
	Man-4	4.208	4.206	4.219
	Man-4'	4.095	n.d.	4.106
H-3ax	NeuNAcα2→6	1.720	-	-
	NeuNAcα2→3	-	-	1.804
H-3eq	NeuNAcα2→6	2.672	-	-
	NeuNAcα2→3	-	-	2.756
H-4	-3Galβ1→4	4.192	4.181	-
H-5	Fucα1→6	4.126	n.d.	-
	Galα1→3	4.192	4.192	-
CH_3	Fucα1→6	1.208	1.201	-
NAc	GlcNAc-1	2.011/2.017	2.013	-
	GlcNAc-2	2.093	2.090	2.058
	GlcNAc-5	2.072	2.055	2.050
	GlcNAc-5'	2.045	2.048	α2.038,β2.043
	GlcNAc-7	2.080	2.081	2.075
	GlcNAc-7'	2.045	2.037	2.038
	NeuNAc	2.031	-	2.030

[a] I-2 also contained a component with NeuNAcα2→6-linked to Gal-6'. In this table only the chemical shifts belonging to the component with three terminal Galα1→3 residues are quoted. The linkage of the trimannosyl core is given.

[b] In the spectrum a doublet at 4.979 was present, which could so far not be interpreted. It is not yet clear if this doublet belonged to a carbohydrate structure or to a contaminant.

Table 3. *Lectin-binding properties of neutral and charged glycopeptides from fraction B*
The neutral glycopeptides were not bound to DEAE-cellulose (Fig. 2A and B) and represented 17% and 3% of the radioactivity of fraction B (Fig. 1) from NIH 3T3 and a1-1 cells, respectively, metabolically labeled with [^{3}H]GlcN. The charged glycopeptides were eluted from DEAE-cellulose with 4–11 mM and 4–16 mM sodium phosphate buffer, respectively (Fig. 2A and B) and represented ≈30% of fraction B (Fig. 1) of NIH 3T3 and a1-1 cells, metabolically labeled with [^{3}H]GlcN. All details are given in Experimental Procedures and Figs 4 and 5

[^{3}H]GlcN glyco-peptides	Immo-bilized lectin	Radioactivity from cells			
		NIH 3T3		a1-1	
		retarded	bound	retarded	bound
		% total			
A. Neutral	L-PHA	0	57	12	19
	GSI	19	53	17	29
	RCAI	46	20	28	22
	lentil		9		25
	ConA		<1		11
B. Charged	L-PHA	0	72	16	47
	GSI	23	66	25	25
	RCAI	56	21	37	10
	SNA		54		16
	lentil		21		15
	ConA		6		10

binding properties to the immobilized lectins L-PHA and GSI (Table 3). L-PHA–agarose has been reported to bind certain tetraantennary glycopeptides [15] and GSI to bind glycopeptides containing α-Gal residues [17, 18]. The neutral glycopeptides (57%) were bound to L-PHA–agarose (Fig. 4A) and only 10% of this L-PHA-bound fraction bound to ConA–Sepharose and lentil–Sepharose in series. 53% of the neutral fraction bound to GSI–agarose (Fig. 4B) with characteristics as defined by Spiro and Bhoyroo [17] of glycopeptides containing two or more α-Gal residues whereas 19% of the radioactivity was retarded in the position of one α-Gal residues (Table 3). Only 20% bound tightly to RCAI–agarose as would be characteristic of more than one terminal

Fig. 3. *Structures proposed for predominant glycopeptides.* Neutral glycopeptide from (A) NIH 3T3 cells; charged glycopeptides from (B) a1-1 cells and (C) NIH 3T3 cells. The numbers in bold type refer to the positions of the monosaccharides

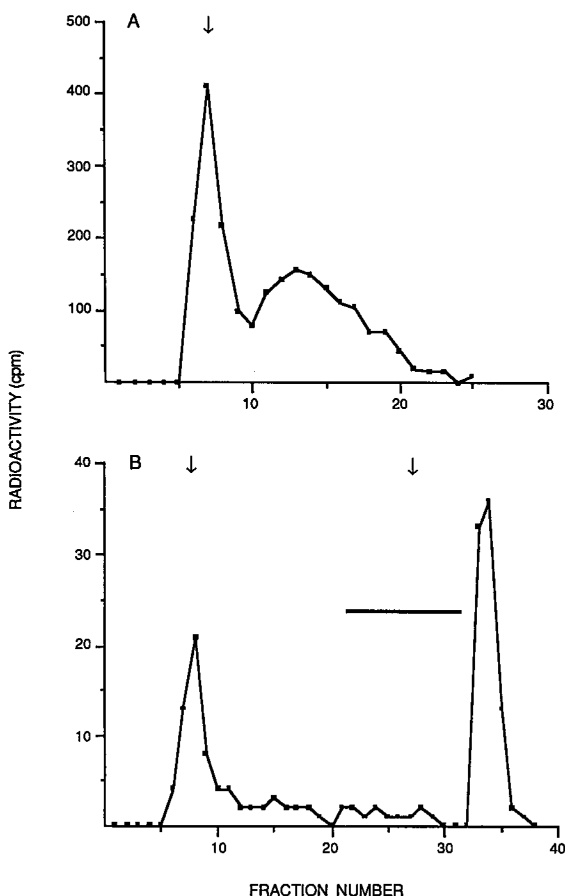


Fig. 4. *Binding of neutral glycopeptides from NIH 3T3 cells to L-PHA–agarose and GSI–agarose.* (A) The neutral glycopeptides (Fig. 2A) of NIH 3T3, after passage over ConA and lentil lectin, were applied to a column (0.5 × 22 cm) of L-PHA–agarose and eluted with buffer [15]; fractions of 0.7 ml were collected. Fractions 11–21 were designated bound (Table 3A). (B) The neutral glycopeptides (Fig. 2A) were applied to a column (0.5 × 22 cm) of GSI–agarose, eluted with buffer and subsequently with 10 mM α-MeGal in buffer [17], as indicated by the right arrow. Fractions of 0.7 ml were collected. The bar marks the elution position of UDP-[^{14}C]Gal, used as internal marker. (A, B) The arrow to the left marks the void volume of the column

β-Gal residues as defined by Campbell and Stanley [19]. However, when the GSI-bound glycopeptides were treated with coffee bean α-galactosidase, 90% of the radioactivity bound tightly to RCAI, demonstrating that the terminal α-Gal residues were linked to β-Gal.

The lack of extensive binding to immobilized ConA and lentil lectin eliminated the presence of specific di- and triantennary glycopeptides which have certain configurations [15, 16]. The lack of affinity for RCAI or binding to DEAE-cellulose showed that few of the glycopeptides had terminal β-Gal or α-NeuNAc residues and furthermore no NeuNAc was seen in the NMR spectrum. Other properties influence weak binding to RCAI–agarose [19] so that the presence of a small number of glycopeptides with one terminal β-Gal residue can not be ascertained even though 46% of the radioactivity was weakly bound to RCAI. Thus, by lectin-binding properties, the predominant glycopeptide was tetraantennary with two or more α-Gal residues compatible with the structure given in Fig. 3A.

Lectin affinity characterization of glycopeptides from a1-1 cells

The neutral glycopeptides from the transformed cells a1-1 (Fig. 2B) were only 7% of fraction B when [^{3}H]Fuc was used as label (Table 1) and not available in sufficient amount for NMR analysis. However, it was clear from the binding properties to L-PHA–agarose and GSI–agarose that these neutral glycopeptides had characteristics different from those of NIH 3T3 cells (Table 3A) and contained no predominant glycopeptide. Only 19% and 29% of the glycopeptides bound to immobilized L-PHA and GSI, respectively. Moreover, there was significant binding to ConA and lentil-lectin–Sepharose compatible with greater heterogeneity and lack of predominant glycopeptides of the tetraantennary type (Table 3A).

CHARGED GLYCOPEPTIDES WITH ONE OR TWO NeuNAc RESIDUES

NMR characterization of the glycopeptides from a1-1 cells

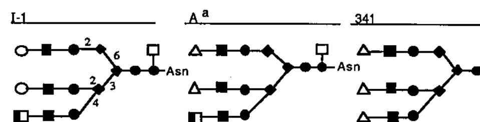
The glycopeptides from the transformed a1-1 cells which were eluted from DEAE-cellulose with 4–16 mM phosphate buffer (Fig. 2B), representing 24% of [^{3}H]Fuc-labeled frac-

Table 4. ^{1}H-Chemical shifts of structural-reporter groups of the constituent monosaccharides for the main component (A) of charged glycopeptides of a1-1.

The fraction of charged glycopeptides were compared to the reference compounds I-1 [29] and 341 (van Pelt and Vliegenthart, unpublished results). Chemical shifts were acquired at 500-MHz for neutral solutions in D_2O at 300 K. Values are in ppm relative to DSS (using internal acetone at 2.225 ppm). See Table 2 for other details and Figure 3 for the numbering of the monosaccharide residues.

●, GlcNAc; ■, β-Gal; ◧, α-Gal; ◆, Man; Δ, NeuNAcα2→3; ○, NeuNAcα2→6; ▫, Fucα1→6.

Compound and schematic structure



Reporter group	Residue	I-1	A^a	341
H-1	GlcNAc-1	5.073/5.053	n.d.	-
	GlcNAc-2	4.681	4.679	5.213
	Man-3	4.77	n.d.	n.d.
	Man-4	5.130	5.116	5.119
	Man-4'	4.932	4.917	4.915
	GlcNAc-5	4.595	4.56	4.560
	GlcNAc-5'	4.602	4.56	4.576
	Gal-6	4.441	4.544	4.546
	Gal-6'	4.447	4.544	4.546
	GlcNAc-7	4.539	4.544	4.546
	Gal-8	4.539	4.544	4.546
	Galα1→3	5.145	5.140	-
	Fucα1→6	4.874	4.870	-
H-2	Man-3	4.216	4.209	α4.226,β4.217
	Man-4	4.216	4.209	4.221
	Man-4'	4.112	4.12	4.112
H-3ax	NeuNAcα2→6	1.717	-	-
	NeuNAcα2→3	-	1.797	1.802
H-3eq	NeuNAcα2→6	2.668	-	-
	NeuNAcα2→3	-	2.757	2.756
H-4	-3Galβ1→4	4.181	4.18	-
H-5	Fucα1→6	4.12	4.12	-
	Galα1→3	4.193	4.19	-
CH_3	Fucα1→6	1.20	1.201	-
NAc	GlcNAc-1	2.008/2.014	2.012	-
	GlcNAc-2	2.093	2.092	2.060
	GlcNAc-5	2.068	2.048	2.046
	GlcNAc-5'	2.066	2.042	2.046
	GlcNAc-7	2.077	2.075	2.074
	NeuNAc	2.029	2.031	2.031

a) The occurence of the terminal α-Gal residue on the Manα1→6 branch cannot be excluded.

tion B and having the charge characteristics of glycopeptides with one or two NeuNAc residues, were examined by high-resolution NMR. The predominant glycopeptide in this charged class gave ^{1}H chemical shifts of structural-reporter groups compatible with a triantennary oligosaccharide (Table 4). Among the interesting structural features of the main glycopeptide in this fraction was the presence of NeuNAcα2→3 and no detectable NeuNAcα2→6, deduced from the H-3 equatorial signal at $\delta = 2.757$ ppm and the H-3 axial signal at $\delta = 1.797$ ppm. The presence of Galα1→3 was inferred from the chemical shift of H-1 at 5.140 ppm and H-5 at 4.19 ppm of this Gal residue. Also the effect on the H-4 of the α1→3-substituted β-linked Gal residues was observed at 4.18 ppm. The triantennary type of structure was deduced from the chemical shifts of the H-2 protons of Man-**3**,-**4**, and -**4'** as well as from the *N*-acetyl signals of the GlcNAc residues. The presence of Fucα1→6 linked to GlcNAc-**1** was seen from the structural reporter group signals of this fucose residue, namely H-1, H-5 and -CH_3 (at 4.870, 4.12 and 1.201 ppm, respectively). The tentative structure of the predominant glycopeptide in this charged fraction from a1-1 cells is given in Fig. 3B. NeuNAcα2→3 has been suggested to residue on the Manα1→6 antennae, although the presence of terminal α-Gal in the Manα1→6 antennae cannot be excluded. It should be noted that the sample also contained a small amount of α2→6 linked NeuNAc together with a biantennary type of structure. However, the amounts of these glycopeptides were too low for a complete structural interpretation.

Other characteristics of the a1-1 glycopeptides

Studies were performed in order to obtain supportive information for the structure of this charged glycopeptide from a1-1 cells. The elution position from DEAE-cellulose was compatible with the presence of one or two NeuNAc residues (Fig. 2B). The lack of binding (84%) of the fraction to SNA—Sepharose was compatible with NeuNAc in α2→3 linkage (Table 3B). SNA lectin has affinity for NeuNAcα-2→6Gal sequences but not NeuNAcα2→3 [20]. Also supportive of the proposed structure was the fact that 50% bound to GSI—agarose, similar to molecules with one or two α-Gal residues (Table 3B).

It was expected that a glycopeptide of the structure given in Fig. 3B would not bind to immobilized lentil lectin or L-PHA since a glycopeptide from thyroglobulin (compound I-1 in Table 4) which contained the same branching pattern around the α-Man residues was shown previously [15] not to bind to these lectins. However, it was subsequently shown that the thyroglobulin glycopeptide was substituted on the Manα1→6 branch with NeuNAcα2→6 [29] whereas the glycopeptide from a1-1 contained NeuNAcα2→3 or Galα1→3 (Table 4). Since 47% of the [^{3}H]GlcN-labeled glycopeptide bound to L-PHA—agarose (Table 3B), it seemed reasonable to examine the binding properties after neuraminidase treatment (Fig. 5A). It was then shown that only 18% of the L-PHA—agarose bound [^{3}H]glycopeptides remained bound to L-PHA (fractions 15—20, Fig. 5B) after neuraminidase treatment and the remaining radioactivity moved to a position slightly retarded (fractions 8—14, Fig. 5B). The [^{3}H]glycopeptides which were changed in their binding properties to L-PHA after removal of sialic acid (fractions 8—14, Fig. 5B) did not bind to GSI—agarose (Fig. 5C), whereas 50% of those which remained bound to L-PHA (fractions 15—20, Fig. 5B) contained one or more α-Gal residues (Fig. 5D). After these studies were completed, it was reported that bind-

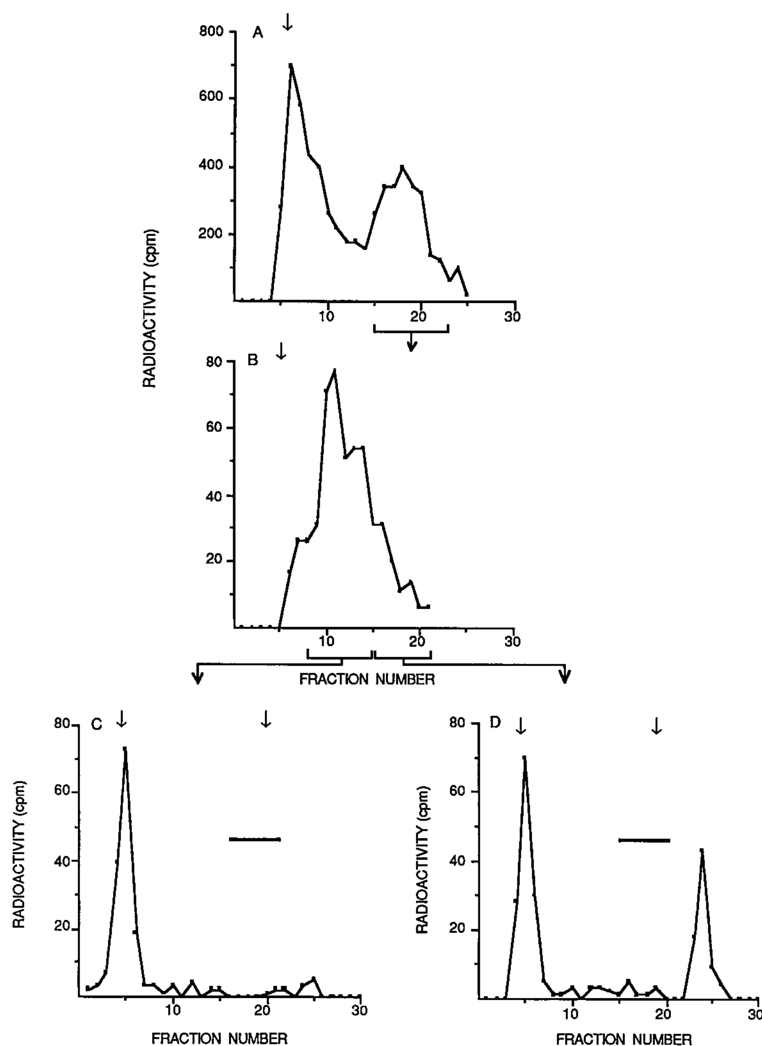


Fig. 5. *Unusual properties on L-PHA–agarose of the charged glycopeptides from a1-1 cells metabolically labeled with [^{3}H]GlcN.* (A) The charged glycopeptides which were eluted from DEAE-cellulose with 4–11 mM phosphate buffer, pH 6.8 (Fig. 2B) were chromatographed on L-PHA–agarose. Fractions of 0.7 ml were collected and fractions 7–14 were designated retarded (Table 3B). The glycopeptides which were bound, fractions 15–26, were treated with *V. cholerae* neuraminidase, desalted on Bio-Gel P-2 and (B) rechromatographed on L-PHA–agarose. (C) Fractions 7–15 and (D) 16–21 of B were examined for binding to GSI–agarose. Fractions of 0.7 ml were collected. (A–D) Arrow on the left designates unbound radioactivity; (C, D) right arrow designates the beginning of the eluting buffer, 10 mM α-MeGal; the bar marks the elution position of UDP-[^{14}C]Gal

ing to L-PHA–agarose was enhanced by oligosaccharides containing α2→3-linked but not α2→6-linked NeuNAc [30]. Thus, this unusual affinity to L-PHA–agarose of the fraction containing this charged glycopeptide was partially supportive of the proposed structure (Fig. 3B).

NMR characterization and other properties of two groups of glycopeptides from NIH 3T3 cells

The charged glycopeptides from NIH 3T3 cells which were eluted from DEAE-cellulose with 4–11 mM phosphate buffer (Fig. 2A) contained 23% of fraction B when [^{3}H]Fuc was the metabolic label. These glycopeptides contrasted with those from a1-1 cells since they contained an equal amount of NeuNAcα2→6 in addition to NeuNAcα2→3. As a result of the NMR studies, NeuNAcα2→6 was suggested to occur on the Manα1→3 branch since the Man-**4** H-1 proton overlapped with the H-1 of Galα1→3 at 5.14 ppm. A NeuNAcα2→3 linked to this branch would give a chemical shift at 5.12 ppm for Man-**4** H-1 [27], and this signal was absent in the NMR spectrum for this fraction. Galα1→3 was present and GlcNAc-**1** was core-fucosylated α1→6. The NMR data however, could not distinguish whether the glycopeptides were tri- or tetraantennary.

The lectin-binding properties of these charged NIH 3T3 glycopeptides showed that 72% of the [^{3}H]GlcN radioactivity bound to L-PHA–agarose whereas only 21% bound to lentil lectin (Table 3B). The binding to L-PHA–agarose was not impaired by treatment with neuraminidase, suggesting a lack of NeuNAcα2→3 on the Manα1→6 branch. NeuNAcα2→3 enhanced the binding of the a1-1 charged glycopeptides (Fig. 3B) to the lectin (Fig. 5). Further studies showed the tetraantennary properties of these L-PHA-bound glycopeptides (Table 5). The glycopeptides contained two or more α-Gal residues as shown by tight binding to GSI–agarose; less than 50% of the radioactive glycopeptides bound tightly to RCAI–agarose, whereas after removal of NeuNAc by neuraminidase, 80% bound to immobilized RCAI. The

glycopeptides (54%) bound to SNA–Sepharose (Table 3B), supporting the presence of NeuNAcα2→6Gal sequences with the remaining glycopeptides containing NeuNAcα2→3 as terminal monosaccharide. On the basis of these results, combined with the position on DEAE-cellulose of a glycopeptide containing one charge, a tentative structure is proposed (Fig. 3C). The NMR data suggests that Gal-**6** is sialylated α2→6. This position has been reported as the primary sialylation site for α2→6-sialyltransferase [31]. By analogy with glycoproteins of known structure [31], NeuNAcα2→3 is tentatively placed on Gal-**8**. Although the precise positions of these sialyl residues need to be determined, the fraction contained a mixture of monosialylated glycopeptides as indicated by the position on DEAE-cellulose. Additional material will be necessary to delineate completely the glycopeptides in this fraction but the predominant glycopeptide is of the tetraantennary type.

Table 5. *Tetraantennary properties of charged NIH 3T3 glycopeptides*
The charged glycopeptides of NIH 3T3 containing 1–2 NeuNAc residues (Table 3B) which were analyzed by NMR were further characterized on L-PHA–agarose. The fractions which were bound to L-PHA–agarose were chromatographed on GSI–agarose and eluted with 10 mM α-MeGal and contained more than one α-Gal residue, whereas the retarded fractions were eluted with buffer and contained one α-Gal residue [17]. The L-PHA-bound fraction was also chromatographed on RCAI–agarose. The fractions which were eluted with 0.1 M lactose contained two or more β-Gal residues [19]. Lectin binding was determined before and after treatment with 0.5 unit *Vibrio cholerae* neuraminidase

Immobilized lectin	[^{3}H]GlcN glycopeptides bound	
	− neuraminidase	+ neuraminidase
	%	
L-PHA	100	100
GSI		
α-Gal (one)	0	0
α-Gal (two)	90	94
RCAI		
β-Gal (one)	0	0
β-Gal (two)	45	81

The next charged group of glycopeptides from NIH 3T3 cells were eluted from DEAE-cellulose with 11–20 mM phosphate buffer (Fig. 2A). The major portion of radioactive glycopeptides in this fraction were eluted with 11–15 mM phosphate buffer and corresponded in charged properties to the first group of charged glycopeptides of a1-1 cells (Fig. 3B) which were eluted with 12–16 mM phosphate buffer. However, NMR analysis revealed an important difference; the presence of NeuNAcα2→6 (≈35%) along with NeuNAcα-2→3 (≈65%) in contrast to the major a1-1 glycopeptide which contained NeuNAc only in α2→3 linkage. The presence of Galα1→3 was detected and, as reported for the other fractions, GlcNAc-**1** was substituted with Fucα1→6.

The presence of Galα1→3 on these charged NIH 3T3 glycopeptides was also shown by affinity to GSI–agarose (Table 6). The glycopeptides (fraction III) had affinity to GSI–agarose, denoting the presence of one or more α-Gal residues on 65% of the glycopeptides. Over 60% of the radioactivity bound to L-PHA, a fact supporting the multiantennary structure.

CHARGED GLYCOPEPTIDES WITH TWO OR MORE NeuNAc RESIDUES

Characterization of highly charged glycopeptides from a1-1 cells

The glycopeptides from a1-1 cells which were eluted from DEAE-cellulose with 16–23 mM phosphate buffer (Fig. 2B) represented 22% of [^{3}H]Fuc-labeled fraction B. These glycopeptides had the charged characteristics of two or more

Table 6. *Inverse correlation between α-Gal residues and charge of glycopeptides*
NIH 3T3 and a1-1 cells were metabolically labeled with L-[^{3}H]Fuc and fractions separated as described in Experimental Procedures. Radioactive fractions which were eluted from DEAE-cellulose (Fig. 2C and D) with a gradient of sodium phosphate buffer, pH 6.8, prior to 16 mM (< 16 mM) or after 16 mM (> 16 mM) sodium phosphate buffer, were collected, desalted, and applied to immobilized GSI. For NIH 3T3 (Fig. 2C), fraction I, 1–12; Ia, 13–25; II, 26–40; III, 41–60; IV-V, 61–109; VI, 110–122. For a1-1 (Fig. 2D), fraction I, 1–16; Ia, 17–28; II-III, 29–49; IV, 50–67; V, 68–91; VI, 108–118. See Table 5 for definition of fractions on GSI

DEAE-cellulose fractions	Radioactivity in		Affinity for immobilized GSI					
	NIH 3T3	a1-1	nonbound		retained		bound	
			NIH 3T3	a1-1	NIH 3T3	a1-1	NIH 3T3	a1-1
	% fraction B		% in fraction					
Neutral								
I	17	5	17	33	17	5	65	64
Ia	7	2	31	43	10	18	58	38
Charged								
<16 mM phosphate buffer								
II	23		24		34		40	
III	38	24	35	56	44	12	21	32
>16 mM phosphate buffer								
IV		22		85		10		5
V	11	22	62	92	26	5	5	3
VI	8	18	76	75	14	13	8	11

NeuNAc residues. NMR spectroscopy showed that the predominant glycopeptides were mainly tri- or tetraantennary. Most interesting was the presence of NeuNAcα2→3 but not NeuNAcα2→6. NeuNAcα2→3 was deduced from the H-3 axial signal at δ = 1.804 ppm and the H-3 equatorial signal at 2.760 ppm. No α-Gal residues were detected and GlcNAc-**1** was substituted with Fucα1→6.

The next charged group of glycopeptides from a1-1 cells were eluted from DEAE-cellulose with 23 – 30 mM phosphate buffer (Fig. 2B) and represented 24% of [^{3}H]Fuc-labeled fraction B. NMR spectroscopy again revealed that the major glycopeptides from the transformed cells contained NeuNAcα2→3 and no NeuNAcα2→6 or Galα1→3. The presence of NeuNAcα2→3 was deduced from the H-3 axial signal at 1.805 ppm and the H-3 equatorial signal 2.758 ppm. GlcNAc-**1** was fucosylated α1→6 as was predicted from the similarity of the DEAE-cellulose profiles from the cells labeled with [^{3}H]GlcN (Fig. 2B) or [^{3}H]Fuc (Fig. 2D). Moreover, the lack of Galα1→3 and NeuNAcα2→6 in both these charged glycopeptide fractions of a1-1 cells was further substantiated by the fact that 85% and 92%, respectively, did not bind to GSI – agarose (Table 6) and 81% and 74% did not bind to SNA – Sepharose. A small amount of diantennary structures were observed in this fraction by NMR spectroscopy in addition to the tri- and tetraantennary glycopeptides. It is assumed they contained a charged group in addition to NeuNAc because of their behaviour on DEAE-cellulose.

Partial characterization of the highly charged glycopeptides of NIH 3T3 cells

In contrast to the glycopeptides of a1-1 cells, only a small amount of radioactivity (<20%) was eluted from DEAE-cellulose with >16 mM phosphate buffer when NIH 3T3 glycopeptides were examined. These glycopeptides were not sufficient for NMR analysis but were examined on GSI – agarose. Approximately 30% still retained α-Gal residues (Table 6) and approximately 60% bound to L-PHA, properties consistent with multiantennary glycopeptides containing one or more α-Gal residues. Moreover, the binding to SNA – Sepharose (62%) was consistent with the presence of NeuNAcα2→6 residues.

Reciprocal relationship of α-NeuNAc and α-Gal

As apparent from the NMR data, the more charged glycopeptides contained less Galα1→3. To examine this relationship quantitatively, the glycopeptides of NIH 3T3 and a1-1 cells, labeled with L[^{3}H]Fuc, were separated on DEAE-cellulose (Fig. 2C and D) and examined for affinity to immobilized GSI. The major portion of the glycopeptides were fucosylated at GlcNAc-**1**; therefore, a direct comparison was made with each fucosyl residue representing a glycopeptide. Note the similarity of the profiles of glycopeptides labeled with either [^{3}H]GlcN (Fig. 2A and B) or [^{3}H]Fuc (Fig. 2C and D) on DEAE-cellulose. In addition, the percentage of total fraction B which had affinity for GSI was similar for each cell type whether the cells were labeled with [^{3}H]Fuc or [^{3}H]GlcN (Table 7). The inverse correlation of α-Gal and α-NeuNAc residues is given in Table 6. In both cell types, the neutral fucosylated glycopeptides contained proportionately more α-Gal residues than the charged glycopeptides. Those glycopeptides which were eluted from DEAE-cellulose in a position indicating two or more NeuNAc residues showed a striking lack of two or more α-Gal residues. In all charged classes, the glycopeptides from NIH 3T3 cells showed a greater affinity for GSI – agarose than did those from a1-1 cells. This was most apparent when the lectin binding of the total B fractions were compared (Table 7). Thus the presence of α-Gal residues in the mouse cells allows a differential of the tumor-forming a1-1 cells from the non-tumor-forming NIH 3T3 cells.

Table 7. *α-Gal residues of glycopeptides from NIH 3T3 and a1-1 cells*
Fraction B (Fig. 1) from NIH 3T3 and a1-1 cells labeled metabolically with L-[^{3}H]Fuc or D-[^{3}H]GlcN was characterized by affinity to immobilized GSI. See Table 5 for definition of affinity to GSI

Number of α-Gal residues	[^{3}H]Fuc in		[^{3}H]GlcN in	
	NIH 3T3	a1-1	NIH 3T3	a1-1
	% of radioactivity			
None	34	72	31	74
One	30	9	28	12
Two or more	32	16	41	13

Glycosylation changes in the presence of swainsonine

In the presence of swainsonine, the transformed a1-1 cells lost a major property of the transformed phenotype: growth in soft agar [10]. By this criteria the cells were no longer transformed when their glycosylation was perturbed. Therefore, the glycosylation changes produced by swainsonine were examined in more detail. The profiles on Bio-Gel P-10 of the pronase-digested glycopeptides of NIH 3T3 and a1-1 cells, treated with swainsonine for 24 h in the presence of L-[^{3}H]Fuc (data not shown) or D-[^{3}H]GlcN (Fig. 1C and D) were similar to each other. There were no glycopeptides equivalent to fraction B in the swainsonine-treated cells.

The glycopeptide profiles from both cell types after swainsonine treatment were also more similar to each other on the basis of charge as shown by binding to DEAE-cellulose (Table 8). The total glycopeptides which were included in Bio-Gel P-10 (Fig. 1) were compared and only two major groups were discerned, a neutral and a charged group which was eluted with <10 mM sodium phosphate buffer, pH 6.8; that is, 77% and 90% of the glycopeptides from a1-1 and NIH 3T3 cells fell into this category. These results would be expected if hybrid-type glycopeptides were formed as shown in other cell types [9]. From the DEAE-cellulose binding studies, it seemed that at least 50% of the hybrid glycopeptides were sialylated. This was verified by binding to serotonin – Sepharose since almost a half of the [^{3}H]GlcN glycopeptides were bound in both cell types. As predicted for glycopeptides from swainsonine-treated cells [9], the glycopeptides from both cell types bound tightly to ConA – Sepharose (Table 8). In contrast, the striking decrease in binding to L-PHA – agarose of both cell types after swainsonine treatment indicated that tetraantennary oligosaccharides were not synthesized. This finding was also supported by a decrease in binding to GSI – agarose, indicating that most glycopeptides had no α-Gal residues.

All of these characteristics demonstrate that the major glycopeptide formed in both the transformed and non-transformed cells after swainsonine treatment was of the diantennary, high-mannose type containing not more than one

Table 8. *Characterization of glycopeptides form swainsonine-treated cells*
The cells were labeled metabolically with D-[^{3}H]GlcN in the presence of 5 µg swainsonine/ml medium; the glycopeptides which were eluted in fractions 25–52 (Fig. 1C and D) from Bio-Gel P-10 (M_r 5000–1500) were characterized. See Table 5 for description of GSI–agarose and Fig. 4 for a column profile. The binding properties of L–PHA–agarose are shown in Figs 4 and 5

Fraction	Radioactivity in cells	
	a1-1	NIH 3T3
	% total	
DEAE-cellulose:		
neutral	41	46
charged (eluted with phosphate buffer)		
<10 mM	36	44
>10 mM	18	8
Serotonin–Sepharose bound	54	47
L-PHA–agarose bound	5	3
ConA–Sepharose bound:		
10 mM α-MeGlc	30	30
200 mM α-MeMan	53	50
GSI–agarose bound[a]:		
buffer	10	11
10 mM α-MeGal	1	3

[a] Neutral fraction from DEAE-cellulose.

NeuNAc residue. Since swainsonine reversed the transformed phenotype of a1-1 cells [10], these studies are strongly supportive of the necessity of sialylated tri- and tetraantennary oligosaccharides for full expression of transformation. One NeuNAc residue on the Manα1→3 antennae does not appear to be sufficient.

DISCUSSION

^{1}H-NMR analysis of the oligosaccharides from the glycoproteins of NIH 3T3 cells has revealed that the major glycopeptides contain those of the tetraantennary type (Fig. 3A). Since these cells do not have the transformed phenotype [12], the results are interpreted as showing that increased branching *per se* is not an accompanying factor of complete transformation of NIH 3T3 cells. These tetraantennary glycopeptides were terminated in α-Gal residues, allowing a ready identification of multiantennae. After transformation by transfection with human DNA containing the H-*ras* oncogene the α-Gal residues were reduced and NeuNAc residues were prominent (Table 4 and Fig. 3). Moreover, the sialyl residues after transformation were in α2→3 linkage whereas NIH 3T3 contained in addition NeuNAcα2→6. Thus, when the NIH 3T3 cells express properties of transformation, the multiantennary oligosaccharides must be specifically sialylated.

Sialylation *per se*, however, is not sufficient to ensure the expression of the transformed phenotype. Treatment of the transformed cell with swainsonine prevented their growth in soft agar, even though a high proportion of radioactive glycopeptides bound to serotonin and DEAE-cellulose (Table 8), suggesting sialylation of the Manα1→3 branch of the swainsonine hybrid glycopeptide, a diantennary oligosaccharide of the high-mannose type [9]. Therefore it is proposed that clustering of sialyl residues is a prerequisite of the transformed phenotype and the multiantennary oligosaccharides provide a base for the clusters. These clustered sialyl residues may provide a charged oligosaccharide shield for the cell, preventing the binding of necessary growth and stabilizing factors. This can be done in two ways: either by bulk intervention or by alteration of specific growth factor receptors. It has been calculated that two sialylated tetraantennary oligosaccharides will cover most of the protein portion of a globular glycoprotein of 80 kDa with the glycan acting as a protective shield [32]. Removal of the sialylated multiantennary oligosaccharides with a specific glycosylation inhibitor clearly alters the transformed phenotype [10, 11, 33]. In another system, a 'cluster' effect of Gal residues was demonstrated for binding of synthetic oligosaccharides to the hepatic Gal/GlcNAc lectin [34]. The branching mode and flexibility of the arm containing the Gal residues were shown to influence the binding affinity. Moreover, multiantennary oligosaccharides had significantly greater affinity than monoantennary oligosaccharides, even though the number of Gal residues was only increased threefold [35].

Sialylation has been reported previously to be associated with metastatic potential [36–38] and the transformed phenotype [4, 39]. However, in these systems the exact glycan structures of the glycoproteins were not determined. In other systems, the glycopeptides derived from the non-transformed cells were predominantly of the diantennary type, whereas after transformation the isolated glycopeptides contained multiantennae [4, 6, 7, 12]. Thus it was thought that increased branching was necessary for the transformed phenotype. The experiments reported here show that, while multibranched oligosaccharides are necessary, they are not sufficient themselves. The fact that α-Gal residues are prevalent in murine cells [40] provided a serendipitous result to target the oligosaccharides of NIH 3T3. Moreover, NIH 3T3 cells may have gone through a step toward the transformed phenotype. These cells are not, however, completely transformed since they do not form tumors in mice or grow in soft agar [12, 13] (and Glick, unpublished results). In fact, the decrease in α-Gal residues may provide a rapid screening procedure for transformation of NIH 3T3 cells.

The interesting effect of NeuNAc on the binding of the sialylated glycopeptides to L-PHA–agarose (Fig. 5) indicates a possible role for specific NeuNAc residues in a biological system. A glycopeptide from thyroglobulin with the same configuration around the α-Man residues but containing NeuNAcα2→6 (compound I-1, Table 4) did not bind to L-PHA [15]; therefore we conclude that the presence of NeuNAcα2→3 (Fig. 3B) confers a new specificity for binding to this lectin (Fig. 5A). After neuraminidase treatment this glycopeptide (Fig. 3B) had little affinity for the lectin (Fig. 5B) as reported when this oligosaccharide contained NeuNAcα2→6 on the Manα1→3 and α1→6 antennae [29]. After this study was completed, Green and Baenziger [30] reported that enhanced binding to L-PHA–agarose was conferred by the presence of NeuNAcα2→3 on the oligosaccharides. Their finding was independently extended here and by Bierhuizen et al. [41] to glycopeptides.

The apparent reciprocal relationship between α-Gal and α-NeuNAc is presumably related to the competitive action of the glycosyltransferases in the NIH 3T3 cell system. Sialylation of N-linked oligosaccharides occurs with branch specificity [31] following an orderly synthesis of these branches

[42]. The specificity of α2→6sialyltransferase from bovine colostrum has been studied using defined substrates [31], it was found that this enzyme showed a preference for Gal-**6** on the Manα1→3 antenna (see Fig. 3C for numbered positions of Gal). By analogy, the oligosaccharides which are sialylated after transformation as reported here, have structures which indicate apparently changed specificities of α2→3sialyltransferase along with an almost complete repression of α-2→6sialyltransferase. In addition, it appears that prior to transformation α1→3galactosyltransferase competes successfully with the sialyltransferases. While other cell systems may be different [5, 8, 11], transformation of NIH 3T3 cells is accompanied by a change in glycosyltransferases responsible for the termination of antennae rather than those conferring the branches. The lack of NeuNAcα2→6 in the transformed cells suggests a possible relationship of α2→3sialyltransferase with transformation of NIH 3T3 cells.

The technical assistance of Ms Florence Massey is acknowledged. Supported by grants from National Institutes of Health (CA 14489 and CA 37853), the Netherlands Foundation for Chemical Research (SON/NWO), the Netherlands Foundation for Cancer Research (KWF-UUKC-OC 83-13) and NATO Grant 0512/87.

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