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Changes in Surface Glycopeptides After Malignant Transformation of Rat Liver Cells and During the Regression of Hepatoma Cells

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Normal liver cells, Zajdela's hepatoma cells, and regressing hepatoma cells were metabolically labeled with either radioactive glucosamine or mannose. Glycopeptides obtained by exhaustive pronase digestion of these cells were compared after fractionation by gel filtration on Bio-Gel P-6.

Chemical analysis, affinity chromatography on immobilized lectins, alkaline treatment, and susceptibility toward endo-β-N-acetylglucosaminidase and tunicamycin revealed dramatic changes in the glycopeptide patterns of transformed cells during the recovery of normal phenotype.

The most prominent feature was the presence on the surface of hepatoma cells of a large glycopeptide, which was absent from normal liver cells and disappeared almost completely during the regression of hepatoma cells. This large glycopeptide had a M_r of 70,000, contained essentially O-glycosidically linked glycan chains, and did not result from a hypersialylation.

N-glycosidically linked glycopeptides, high-mannose, and complex-type oligosaccharides were present in distinct proportions according to the differentiation state. Transformation of liver cells led to a reduction of high-mannose type oligosaccharides and an increase in the degree of branching of complex-type oligosaccharides. In addition, "bisected" glycopeptides were present only on hepatoma cells. The pattern of N-linked glycopeptides of normal liver cells was recovered during the regression of hepatoma cells.

The origin of glycopeptide differences between normal and transformed cells and the evidence of a relation between carbohydrate changes, in particular the appearance of a large glycopeptide, and tumorigenicity are discussed.

Key words: glycopeptides, liver cells, malignancy, malignant transformation, regression, surface glycoproteins

It is well established that the carbohydrate moieties of surface glycoconjugates are involved in ektobiological activities of cells such as cell-cell recognition, adhe-

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siveness, control of cell division, cellular differentiation, malignant transformation [1–4], as well as in the escape of tumors from immune surveillance [5].

Modifications of the biological properties associated with malignant transformation influence intercellular recognition and cell-cell adhesion and could result from alterations in the composition, structure, topography, and/or dynamics of cell surface glycoproteins [6–11].

The carbohydrate moieties of glycoconjugates of malignant cells differ in several aspects from their normal counterparts [9–15]; however, the changes observed in a variety of tumor systems are rather inconsistent, except that malignant cells exhibit an increase of large glycopeptides compared to homologous normal cells. Nevertheless, to date no strict relation between changes in the structure of surface glycoconjugates and cell tumorigenicity has been evidenced, although transformed cells that undergo these characteristic changes are tumorigenic in appropriate hosts [16–17].

Thus, a molecular analysis of cell surface glycoconjugates seems of importance to give a basis for malignancy and to understand the biological properties of malignant cells.

In this work, we have extended previous findings on cell surface glycopeptides of normal and hepatoma cells [18–19] to a more detailed level. In addition, relations between different phenotypes and glycopeptide structure were investigated throughout the regression of hepatoma cells to the normal phenotype. The results obtained show that the differences between glycopeptides of normal and transformed cells are both quantitative and qualitative and support a correlation between changes in oligosaccharide structures and malignant transformation.

EXPERIMENTAL PROCEDURES

Cells and Cell Cultures

Tumor cells. Zajdela's ascites hepatoma is an undifferentiated tumor transplanted in rat and constitutes one of the most rapidly growing tumors in animals [20]. Tumor cells were maintained by serial intraperitoneal transplantation of tumor ascites (0.3 ml/animal) in 6- to 9-week-old male Sprague-Dawley rats (Charles River, Elbeuf, France). Cells were harvested 5–8 days following inoculation and were washed three times in Dulbecco's phosphate-buffered saline (PBS: KH_2PO_4 200, NaH_2PO_4 1,150, KCl 200, and NaCl 8,000 mg/liter), pH 7.4. Centrifugation at 55g for 5 min permitted the isolation of tumor cells with a minimal contamination by erythrocytes and lymphocytes. Hepatoma cells (5×10^5 cells/ml) were cultured in minimum essential medium (MEM) (Gibco, Grand Island, NY) added with 5% fetal calf serum and antibiotics under a humid atmosphere containing 5% CO_2.

Normal liver cells. A suspension of enzymatically dispersed liver cells was prepared by the collagenase perfusion technique described by Seglen [21] and modified by Guguen-Guillouzo et al [22]. Briefly, livers of 150- to 300-g male Sprague-Dawley rats were perfused with 200 ml of Hepes buffer, pH 7.65, and then with 200 ml of collagenase solution (Boehringer, Nannheim, FRG, 0.5 mg/ml in the same Hepes buffer). After perfusion, the liver was removed, and cells were isolated. Cells were then washed three times with Hepes buffer and once with the culture medium. Each washing was performed with 5 ml for 10^5 cells at room temperature and centrifuged at 300g for 1 min. Normal liver cells (2×10^7) were cultured in a 175-cm^2 flask in

20 ml of complex medium containing insulin (10 mg/liter), hydrocortisone (7×10^{-5} M) and 10% fetal calf serum.

Regressing hepatoma cells. These cells were obtained by in vitro culture of Zajdela's hepatoma cells in MEM H 16 medium (Gibco) containing glucose (8 g/liter) and 5% fetal calf serum [23]. These cells adhere to culture flask after a few days. The culture was then treated with TPCK-trypsin (Worthington, Freehold, NJ, 224 units/mg) at the concentration of 50 μg/ml at 37°C for 10 min, and cells were replated in the same medium each week. After about ten passages, a stable phenotype was obtained (Z_{10}) which recovered morphological and certain biochemical properties of normal cells, particularly the glycogenogenesis function [23]. Z_5 represents the 5th passage, when the stable phenotype is not completely established.

Metabolic Labeling of Glycopeptides

The labeled precursors were obtained from Amersham International (Bucks, UK): D-[6-^{3}H]glucosamine (55 Ci/mmol), D-[2-^{3}H]mannose (10 Ci/mmol), and [^{35}S]sodium sulfate (1,160 Ci/mmol). They were added to cultures of normal and tumor liver cells at the concentration of 2.5, 10, and 4 μCi/ml, respectively, and the cultures were continued for 24 h. The cell monolayers of normal and regressing cells were first rinsed three times with PBS, pH 7.4, then scraped off with a rubber policeman. Finally, these adherent cells and the tumor cells were washed three times with PBS and centrifuged at 55g for 5 min in PBS, pH 7.4.

Inhibition of Glycosylation—Action of Tunicamycin

Tunicamycin (Glaxo Group Research Ltd, London, UK), an inhibitor of N-glycosylation [24], was added to the culture medium at final concentration of 1 μg/ml. After 4 h, the radiolabeled precursors were added, and incorporation was continued for 24 h. A parallel control culture without tunicamycin was carried out under the same conditions.

Preparation of Glycopeptides and Resolution by Gel Filtration

Lipids were extracted from labeled cell pellets with 5 ml of a chloroform-methanol mixture (2/1, v/v) for 20 min at 20°C, then with 5 ml of a chloroform-methanol-water mixture (10/10/3, v/v/v) for 20 min at 20°C. The suspension was finally centrifuged for 5 min at 200g.

Delipidated cells were suspended in 0.05 M Tris-HCl buffer, pH 7.8 containing 2 mM $CaCl_2$ and treated with pronase (Calbiochem-Behring Corp, Narburgh, 1 mg/10 mg of protein). The mixture was incubated at 60°C for 24 h under a toluene layer. Then pronase at the same concentration was added for a second 24 h-period of proteolysis. The proteolysis product was centrifuged for 5 min at 200g, and the supernatant was lyophilized.

The lyophilizate was dissolved in 1 ml of 0.1 M pyridine-acetate buffer, pH 5.2, and applied to a Bio-Gel P-6 column (Bio-Rad Laboratories, Richmond, VA, 200–400 mesh (120 × 1 cm), which was equilibrated and eluted with this same buffer. Fractions (1 ml) were collected at a flow rate of 4 ml/h, and 0.1-ml aliquots were assayed for radioactivity. Radioactivity was measured by solubilizing samples with 3 ml of scintillation liquid (ACS) and counted in an intertechnique SL 400 spectrometer. Radioactive fractions were pooled and lyophilized.

Bio-Gel P-6 column was calibrated using bovine serum albumin, [^{3}H]mannose, labeled transferrin, and fetuin glycopeptides of $M_r \simeq 2{,}990$ and 2,320 respectively (kindly supplied by Dr. Finne, The University of Helsinki, Finland). Molecular weights were estimated from the partition coefficients (K_D).

Sepharose 6B column (Pharmacia, Uppsala, Sweden) (120 × 1 cm) was equilibrated and eluted with 0.1 M pyridine-acetate buffer, pH 5.2. Fractions (1.2 ml) were collected and assayed for radioactivity.

Bio-Gel P-4 column (Bio-Rad Laboratories, minus 400 mesh) (200 × 1.2 cm) was equilibrated in 0.1 M pyridine-acetate buffer, pH 5.2. Fractions (0.5 ml) were collected and assayed for radioactivity. The column was calibrated with a dextran hydrolysate according to the method described by Yamashita et al [25] and with $Glc_3Man_9GlcNac$ and $Glc_2Man_9GlcNac$ oligosaccharides prepared from chicken liver [26].

Affinity Chromatography in Immobilized Lectins

A Con A-Sepharose column (Pharmacia, Sweden, 0.7 × 5 cm) was equilibrated in 5 mM sodium acetate buffer, pH 5.2 containing 1 mM $CaCl_2$ and 1 mM $MnCl_2$. The glycopeptide fraction dissolved in this buffer was applied to the column, which was then eluted with 0.1 M NaCl in the equilibration buffer. Elution of bound glycopeptides was performed with 10 mM α-methyl glucoside then 200 mM α-methylmannoside (Sigma, St. Louis) in the equilibration buffer containing 0.1 M NaCl [27]. Fractions (1 ml) were collected and analysed for radioactivity.

E-PHA-Sepharose column (E.Y. Laboratories, USA, 0.5 × 30 cm) was equilibrated in PBS, pH 7.4, containing 1 mM $CaCl_2$ and 1 mM $MgCl_2$. The glycopeptide fraction dissolved in Ca^{++}-free buffer was applied to the column. Elution was then carried out with the equilibration buffer. Fractions (0.5 ml) were collected and analysed for radioactivity [28].

Enzyme Treatments

Endo-β-acetylglucosaminidase H from *Streptomyces griseus* (Seikagaku Kogyo Co Ltd, Japan). Glycopeptides were dissolved in 50 mM sodium citrate buffer, pH 5.0, and incubated with 20 mU of enzyme/ml for 16 h at 37°C [29]. Digestion was terminated by boiling for 3 min.

α-Mannosidase from *Canavalia ensiformis* (Boehringer, Nannheim, FRG). Glycopeptides were dissolved in 0.4 M ammonium acetate buffer, pH 4.5, and incubated with 1 IU of enzyme/ml for 16 h at 37°C. The digestion was stopped by boiling for 3 min.

Neuraminidase from *Vibrio cholerae* (Behringwerke, Narburgh, FRG). Glycopeptides were dissolved in 50 mM ammonium acetate buffer, pH 5.5, containing 10 mM $CaCl_2$ at 37°C and incubated with 1 IU of enzyme/ml for 16 h at 37°C. The digestion was stopped by boiling for 3 min.

Hyaluronidase (Sigma, 15,000 IU/mg). Samples were dissolved in 50 mM ammonium acetate buffer, pH 6.0, and incubated with 300 IU of enzyme/ml for 24 h at 37°C.

Alkaline Treatment

Labeled glycopeptides were treated with 1 ml of 1 M $NaBH_4$ in 0.1 M NaOH at 37°C for 16 h in the dark [30]. The solution was then chilled at 0°C, and the

excess of borohydride was destroyed by dropwise addition of 4 M acetic acid up to pH 5.0. The oligosaccharide solution was resolved by filtration on a Bio-Gel P-6 column.

Identification of Radiolabeled Sugars

This was performed by paper chromatography (Schleicher & Schüll 2043 B paper) in butanol/pyridine/0. 1 N HCl (5/3/2, v/v/v) after hydrolysis of labeled cells at 100°C in 2 N HCl for 2 h or in 3 N HCl for 4 h to release hexoses and hexosamines, respectively. A control monosaccharide mixture was treated under the same conditions and revealed by $AgNO_3$ reagent. The hydrolysate band was cut in 0.5-mm strips, which were added to 3 ml of scintillation liquid, and radioactivity was determined.

After [^{3}H]mannose incorporation, radioactivity was exclusively found in mannose in the different cell types, whereas, after [^{3}H]glucosamine labeling, radioactivity was recovered in both glucosamine and galactosamine with ratios varying in each class of glycopeptides. A very weak conversion of glucosamine (about 10%) into sialic acid was observed.

Analytical Methods

Carbohydrate analysis was carried out by gas liquid chromatography (Hewlett-Packard model 5710 A) of pertrimethyl silylated derivatives of methylglycosides formed by methanolysis in methanol/1.5 M HCl at 85°C for 18 h [31]. Mannitol was used as an internal reference.

RESULTS

Essentially two types of glycopeptides were separated by gel filtration chromatography of radiolabeled glycopeptides obtained from normal and hepatoma cells. Considerable differences were observed according to the differentiation state of the cells and to the radiolabeled monosaccharide precursor used (Fig. 1A, B).

High Molecular Weight Glycopeptides (Peak I)

One class of glycoconjugates (peak I) was excluded by gel filtration on Bio-Gel P-6. Glucosamine-labeled normal and tumor liver cells exhibit dramatically different proportions of these high glycopeptides since they represent 60% of the total glucosamine radioactivity in tumor cells and only 20% in normal cells.

Incomplete pronase digestion did not account for the high molecular weight of these glycoconjugates since elution profiles were not modified after an additional pronase incubation. It could not be due to the presence of glycolipids because cell pellets were previously delipidated. Furthermore, the very low incorporation of [^{35}S]sodium sulfate and the unmodified size of peak I after hyaluronidase and neuraminidase treatments indicated that neither glycosaminoglycans nor sialic acid were responsible to this high molecular weight.

Further fractionation upon Sepharose 6B resolved this class of glycopeptides into three peaks different in size and structure (Fig. 2A, B). Peak IA, which was present exclusively in hepatoma cells, displayed a very high molecular weight ($M_r \simeq$ 70,000). It was characterized by the presence of galactosamine and the absence of

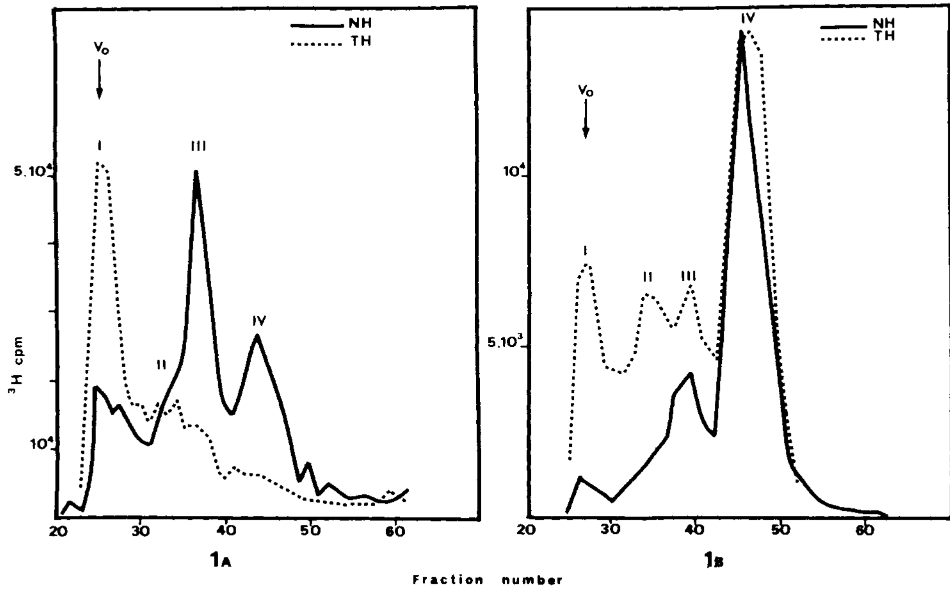

Fig. 1. Gel filtration on Bio-Gel P-6 (200–400 mesh) of glycopeptide fractions obtained from normal (NH) and tumor (TH) liver cells by pronase hydrolysis after [^{3}H]glucosamine (**A**) and [^{3}H]mannose (**B**) incorporation. Column dimensions 120 × 1 cm; 0.1 M pyridine acetate buffer, pH 5.2; flow rate 4 ml/h; each fraction contains 1 ml.

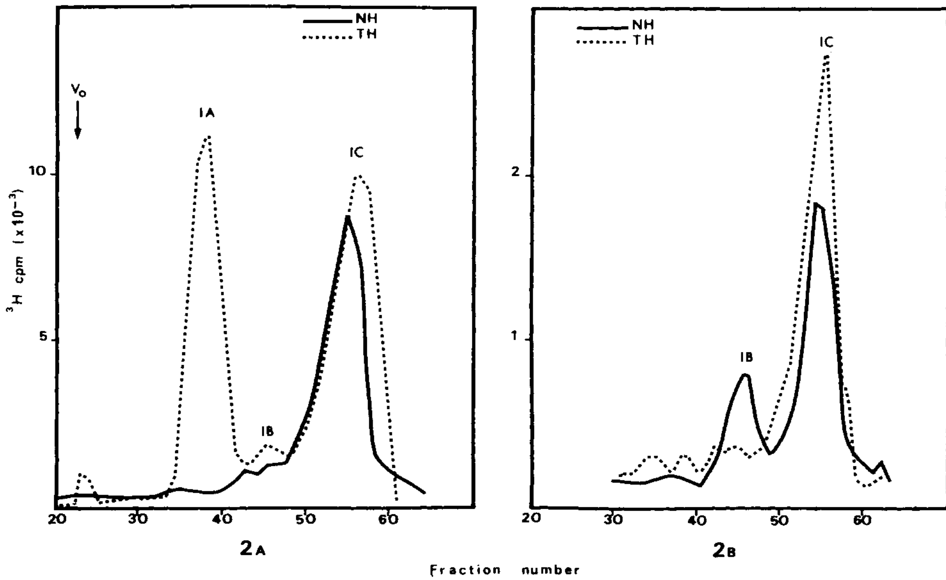

Fig. 2. Gel filtration on Sepharose-6B of fractions I excluded by chromatography on Bio-Gel P-6 (see Fig. 1) and obtained from normal (NH) and tumor (TH) liver cells. **A**: [^{3}H]glucosamine labeling. **B**: [^{3}H]mannose labeling. Column dimensions 120 × 1 cm; 0.1 M pyridine acetate buffer, pH 5.2; flow rate 3.5 ml/h; each fraction contains 1.2 ml.

TABLE I. Chemical Composition of Glycopeptide Fractions From Normal and Hepatoma Cells*

	Fraction no.									
	IA		IC		II		III		IV	
	NH	TH	NH	TH	NH	TH	NH	TH	NH	TH
Galactose	None	35	12	35	5	8	10	5	3	2
Mannose	None	None	4	8	6	10	12	6	30	20
Glucosamine	None	17	15	30	8	17	20	10	11	7
Galactosamine	None	15	3	None	None	None	None	None	None	None
Sialic acid	None	30	7	10	5	4	7	5	None	None
Fucose	None	None	0.7	1.3	2	3	3.1	1.7	3.3	0.7

*Labeled cell pellets after lipid extraction and pronase digestion were chromatographed on Bio-Gel P-6. Peaks I (excluded from Bio-Gel P-6) obtained from the different cell types were then applied to a Sepharose 6B column. Fractions (1 ml) were collected and assayed for radioactivity. The fraction Nos. are those indicated in Figures 1 and 2, and results are expressed in $\mu g/10^8$ cells.
NH, normal liver cells; TH, hepatoma cells.

mannose (Table I) and by a susceptibility to alkaline treatment. Its biosynthesis was resistant to tunicamycin. These data suggest that this large glycopeptide is essentially constituted of O-glycosidically linked glycan chains. Peak IC, present in both cell types, was characterized by a high content of galactose and glucosamine with respect to a low content of mannose (Table I). On the basis of its high molecular weight, characteristic chemical composition, and inhibition of biosynthesis by tunicamycin, this peak appears to be essentially constituted of N-linked lactosaminoglycan chains. Peak IB, available in too small quantities, was not studied.

Asparagine-Linked Glycan Chains (Peaks II–IV)

These N-linked glycopeptides could be discriminated in two types:

High-mannose type (peak IV). This fraction consisted of glycopeptides of molecular weight in a range of 1,500–2,000, the chemical composition of which was characterized by a high content of mannose and the absence of galactose and galactosamine (Table I). In addition, these glycopeptides 1) were completely hydrolysed into high-mannose oligosaccharides by endo-β-N-acetylglucosaminidase H, 2) released mannose upon α-mannosidase treatment, 3) were totally insensitive to alkaline treatment, 4) were strongly bound to Con-A-Sepharose column and only eluted with 0.2 M α-methyl mannoside, 5) had a biosynthesis that was totally inhibited by tunicamycin. These data thus indicate that these glycopeptides are of high-mannose type.

Five high-mannose oligosaccharides containing 9 to 5 mannose residues and 1 glucosamine residue were fractionated by gel filtration on Bio-Gel P-4 (Fig. 3). Although they were present in similar proportions in both cell types, on the basis of total mannose incorporation, this fraction appeared more important in normal than in hepatoma cells (Fig. 1B).

Complex N-linked glycopeptide type (peaks II and III). These glycopeptides displayed a molecular weight in the range of 2,300–4,000 with features characteristic of complex N-linked glycopeptides. Indeed, 1) they contained mannose but no galactosamine, 2) they were susceptible neither to alkaline nor to endo-β-N-acetylglucosaminidase H treatments, and 3) their biosynthesis was almost totally inhibited by tunicamycin.

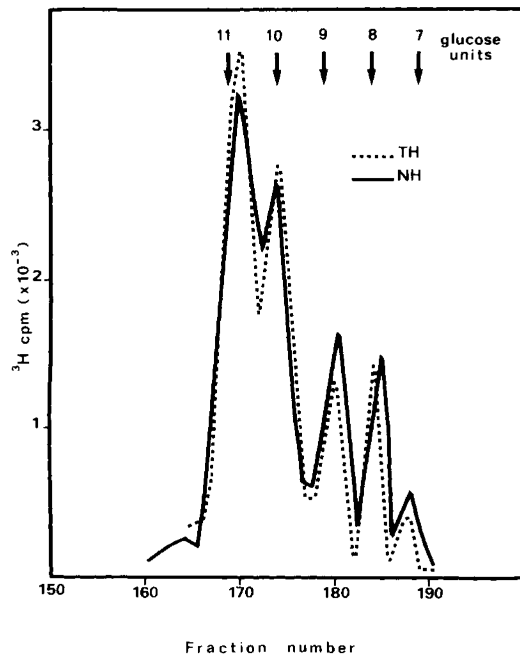

Fig. 3. Gel filtration on Bio-Gel P-4 (minus 400) of fractions IV obtained by Bio-Gel P-6 chromatography after endo-β-N-acetylglucosaminidase H hydrolysis. [^{3}H]mannose was used as radioactive precursor in normal (NH) and tumor (TH) liver cells. Column dimensions 200 × 1.2 cm; 0.1 M pyridine acetate buffer, pH 5.2; flow rate 13 ml/24 h; each fraction contains 0.5 ml. The column was calibrated with a dextran hydrolysate. Elution fractions of oligosaccharides containing 6–10 glucose units were indicated by arrows.

By affinity chromatography on immobilized lectins, it was shown that peak II in both cell types contained only N-linked chains that are more complex than biantennary chains because they were unbound to Con-A-Sepharose. Peak III showed large differences between normal and tumor cells, particularly in the branching degree. Normal cells contained 70% of biantennary and 30% of highly branched glycopeptides, whereas tumor cells contained only this latter type of glycopeptides (Fig. 4). From affinity chromatography on E-PHA-Sepharose, it could be suggested that 30% of the highly branched glycopeptides in tumor cells had an additional glucosamine residue β(1→4) linked to the innermost mannose residue giving "bisected type glycopeptides." This glycopeptide type was absent from normal cells. Thus, tumor cells contain more highly branched glycopeptides than normal cells.

Changes of Glycopeptide Patterns During Hepatoma Cell Regression

In order to know whether these considerable molecular differences of glycosylation between normal and tumor cells may be related to malignant cell transformation, the glycopeptide profiles of these cells were compared with those of hepatoma cells that had partially redifferentiated toward a normal phenotype.

Globally, a progressive return to normal glycopeptide patterns was observed throughout redifferentiation of tumor cells. The proportions of high-mannose glycopeptides increased, and one of the complex N-linked glycopeptides decreased during regression; Z_{10} cells reached values observed in normal cells (Table II). However,

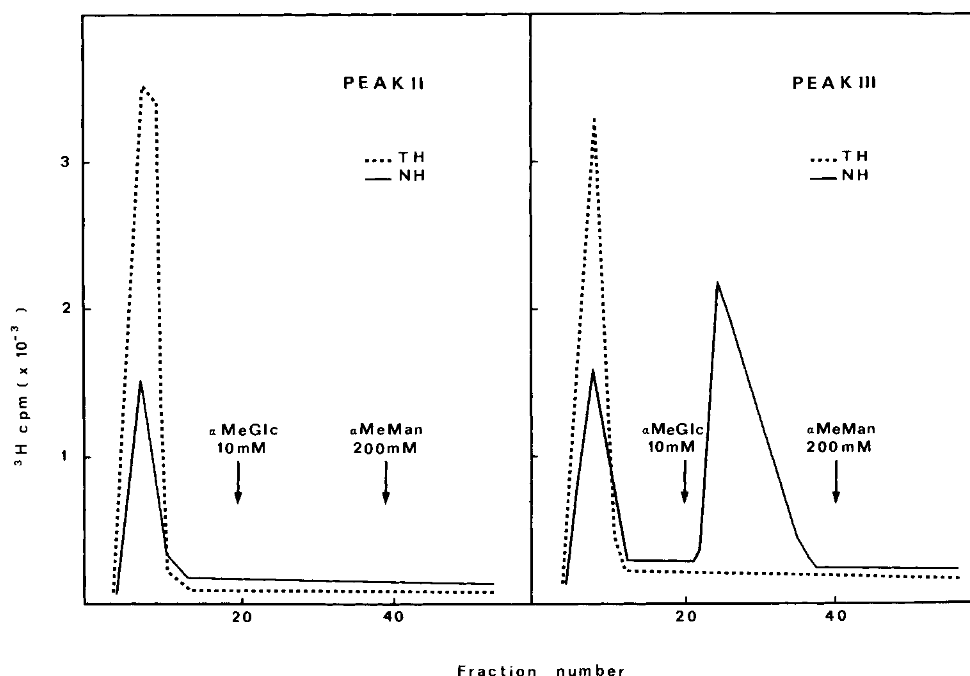

Fig. 4. Con A-Sepharose affinity chromatography of [^{3}H]mannose-labeled glycopeptide obtained from normal liver cells (NH) and hepatoma cells (TH) by pronase digestion. Column dimensions: 5 × 0.7 cm. Arrows indicate the points at which eluant sugar solutions were applied.

TABLE II. [^{3}H]Mannose Radioactivity Repartition (%) in the Three Glycopeptide Classes for the Different Cell Types*

Peak no. on Bio-Gel P-6 on Sepharose 6B	High molecular weight: I[a] IA[b]	IC[b]	N-Complex: II + III[a]	High-mannose: IV[a]
Hepatoma cells	None	17	38	45
Z_5 cells	None	10	37	53
Z_{10} cells	None	10	25	65
Normal liver cells	None	5	26	69

*Labeled cell pellets after lipid extraction and pronase digestion were chromatographed on Bio-Gel P-6. Peaks I (excluded from Bio-Gel P-6) obtained from the different cell types were then applied to a Sepharose 6B column. Fractions (1 ml) were collected and assayed for radioactivity.
[a]Peak No. on Bio-Gel P-6.
[b]Peak No. on Sepharose 6B. Peak IA does not incorporate [^{3}H]mannose.

the most dramatic event was the decrease and then the "quasi" disappearance of the high molecular weight glycopeptide IA, characteristic of hepatoma cells (Fig. 5).

It appears, therefore, that partial redifferentiation of Zajdela's tumor cells is accompanied by an almost complete return to a normal glycopeptide pattern.

DISCUSSION

It is clear that considerable qualitative and quantitative differences occur in the biosynthetic pathway of protein glycosylation in Zajdela's hepatoma cells compared

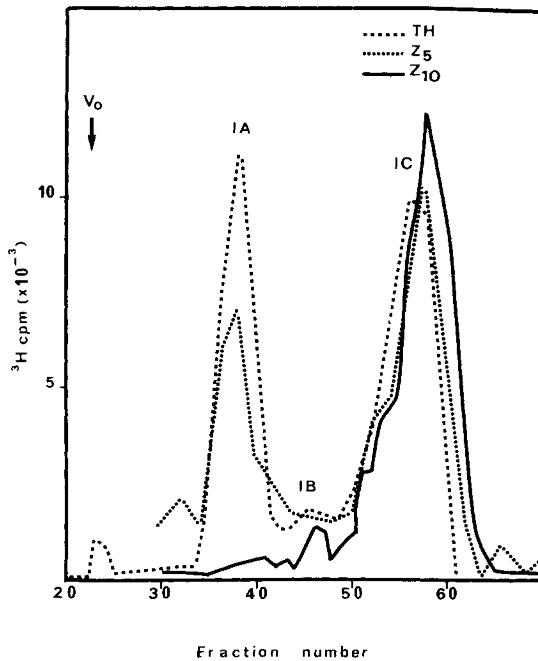

Fig. 5. Gel filtration on Sepharose-6B of fractions I excluded by chromatography on Bio-Gel P-6 and obtained from TH, Z_5, and Z_{10} cells. [^{3}H]glucosamine was used as radioactive precursor in tumor (TH) and "regressing" liver cells at two stages of reversion, fifth passage (Z_5), and more than tenth passage (Z_{10}). Conditions as shown in Figure 2.

to normal liver cells. These carbohydrate changes are largely reversed when, under certain culture conditions, hepatoma cells lose their undifferentiated character and partially recover the properties of the normal phenotype. These differences reflect actual changes in carbohydrate moieties exposed at the cell surface since whole cells as well as plasma membranes digested by pronase give identical glycopeptide patterns. This has been observed in other cell systems [32,33].

The most prominent characteristic is the presence in hepatoma cells of a macroglycopeptide of high molecular weight ($M_r \simeq 70{,}000$), made up of O-linked glycan chains, probably combined in clusters. This unusual carbohydrate structure, which is not detected in normal liver cells, gradually disappears when hepatoma cells regress to the normal phenotype. The presence of large glycopeptides in undifferentiated cells, which are absent or present in considerably diminished amounts in their normal counterparts, has been reported in various cell systems [9–11]. However, the structure of these large glycopeptides seems to differ according to the cell type as well as to the culture conditions, ie, in suspension or in monolayer. The increased molecular weight of transformed cell glycopeptides has been mostly attributed to a higher content of sialic acid in the glycan chains [9,12–13,34]. This hypersialylation would be induced by a specific sialyl-transferase [34]. However, these large glycopeptides do not always result from additional sialic acid residues [35,36]. They are of the mucin type ($M_r \simeq 300{,}000$) with numerous O-linked glycan chains in epiglycanin, a membrane glycoprotein present in tumorigenic 3T3 Ha cells, but not in nontumorigenic 3T3 St cells [37,38]. They are of the N-linked lactosaminoglycan type ($M_r \simeq$

10,000–20,000) in embryo carcinoma cells and disappear when these cells are differentiating [39]. Although large glycopeptides have been revealed in several ascites hepatoma cells, they remain uncharacterized [18,40–42]. This work indicates that at least in Zajdela's hepatoma cells, large glycopeptides are of the mucin type, just as in epiglycanin, and that sialic acid is not responsible for this high molecular weight.

From these data and from the presence of large glycopeptides only at permissive-temperature in cells transformed by a temperature-sensitive virus mutant [43], there are considerable evidences that the appearance of these glycopeptides is a general phenomenon detected in transformed cells independently of cell species and of the mode of transformation. It was therefore tempting to anticipate that the appearance of large glycopeptides is highly correlated with tumorigenicity, and to consider these molecules as associated with the expression of transformed phenotype [14,16–17].

In our system, quantitative and qualitative structural changes occurred in N-linked glycopeptides during cell transformation. High-mannose glycopeptides were reduced during liver cell transformation (this work) and virus transformation [44]. This could be explained by an accelerated trimming of mannose residues leading to the biosynthesis of complex oligosaccharide chains. In contrast, others showed that oligomannosyl chains are generally related to an undifferentiated and growing state [45], which suggested that there is no close relation between the relative amounts of this class of glycopeptides and whether the cells are normal or tumorigenic. These glycopeptides would be implicated in cell-cell interactions in developing systems [46].

The complex N-linked glycopeptides of normal and hepatoma cells differ in their branching degree. As observed in several normal/transformed cell systems [35–36,47], normal liver cells have a limited capacity to biosynthetize highly branched outer chains compared to hepatoma cells, in which transformation leads to an increase of tri/tetraantennary oligosaccharides. The step of N-acetylglucosaminylation appears as the key to the structural changes of N-linked oligosaccharides in transformed cells so that these latter acquire an enhanced capacity to transfer one or more molecules of N-acetylglucosamine to mannose residues already linked to glycoproteins in the endoplasmic reticulum and the Golgi membranes. Another important difference is that "bisected type" glycopeptides were detected in 30% of N-linked highly branched glycopeptides in hepatoma cells but that this glycopeptide type was absent from normal cells. Probably normal liver cells do not contain the enzyme responsible for the addition of bisecting GLcNAc residue and cell transformation induced this enzyme. A similar difference was observed in carbohydrate chains of γ glutamyltranspeptidase from rat liver and rat AH-66 hepatoma cells [48].

The causes of the multiple and profound changes in glycosylation during cell malignant transformation are still hypothetical. They may be related to the expression or repression of mRNA inducing biosynthesis of particular polypeptide chains and/or post-transcriptional changes in protein glycosylation. The altered compartimentalization and the derepression of gene(s) coding for glycosyl transferases as a result of malignancy could also account for these structural differences exerted on the same polypeptide chain expressed by both normal and transformed cells. Indeed, important changes in glycosyl transferases during cell transformation have been reported [1,9,11], but the conclusions are often contradictory.

Finally, these differences in oligosaccharide structures of cell plasma membranes resulting from cell transformation may contribute to alter cell behavior, and

results suggest that the glycan moieties contain structural information possibly relevant to essential biological properties of the cells.

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