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ISOLATION AND PARTIAL CHARACTERIZATION OF A MUCIN-TYPE GLYCOPROTEIN FROM PLASMA MEMBRANES OF HUMAN MELANOMA CELLS

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Plasma membranes were isolated from HM7 melanoma cells grown in the presence of [^{3}H]glucosamine and $Na_2^{35}SO_4$ or [^{3}H]mannose and [^{14}C]glucosamine. The labeled glycoconjugates were solubilized with 0.6 M lithium diiodosalicylate/0.5% Triton X-100. Fractionation of glycoconjugates by repeated chromatography on columns of Sepharose CL-6B and DEAE-Sepharose and by affinity chromatography on WGA-Sepharose yielded three radiochemically homogenous glycoproteins. One of these having an apparent molecular weight of 100 000 was found to contain clusters of (AcNeu)$_{1\text{ or }2}$ → [Gal → GalNAc] linked *O*-glycosidically to the protein. One other glycoprotein contained both *O*-glycosidically and *N*-glycosidically-linked oligosaccharides, and the third contained only *N*-glycosidically-linked carbohydrates. Preliminary results indicate that the 100 000 molecular weight mucin-type glycoprotein is present in significantly reduced quantities in cultured human fetal uveal melanocytes. Further, the bulk of the glycoproteins from the melanocytes were of lower molecular size compared to those from the melanoma cells.

Introduction

We recently described the characterization of glycopeptides derived from HM7 human melanoma cells and from human fetal uveal melanocytes [1]. The patterns of glycopeptides obtained from these two sources were strikingly different. The melanoma cells produced increased quantities of the two wheat germ agglutinin (WGA)-binding mucin-type glycopeptides. These glycopeptides were shown to have highly sialylated oligosaccharides linked *O*-glycosidically to protein, in clusters. On the other hand, for the WGA-nonbinding glycopeptides, there was a significantly higher proportion of the smaller molecular size less anionic species derived from melanocytes compared to those from the melanoma cells. Similar increases in the size of the *N*-glycosidic glycopeptides from cancer cells and tumors have been reported by several workers (see review by Warren et al. [2]).

In the present study, we have succeeded in isolating three glycoproteins from plasma membranes of HM7 human melanoma cells. The partial characterization of an acidic mucin-type glycoprotein is discussed. A preliminary report has been presented [3].

Materials and Methods

Materials

The HM7 human melanoma cells [1] and cultured FM13 human fetal uveal melanocytes [4,5] described previously were used in these studies. Pronase CB and *Vibrio cholerae* neuraminidase (EC 3.2.1.18) were

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Abbreviations: WGA, wheat germ agglutinin; AcNeu, *N*-acetylneuraminic acid; GalNAc(OH), *N*-acetylgalactosaminitol.

purchased from Calbiochem (La Jolla, CA). Sepharose CL-6B, DEAE-Sepharose CL-6B and Sephadex G-50 were obtained from Pharmacia (Piscataway, NJ). Endo-α-*N*-acetylgalactosaminidase (EC 3.2.1.97) from *Diplococcus pneumoniae* culture filtrates was prepared in our laboratory [6]. Wheat germ agglutinin was isolated and conjugated as described [7]. D-[6-^{3}H]glucosamine hydrochloride; [1-^{14}C]glucosamine hydrochloride; $Na_2^{35}SO_4$ and D-[1-^{3}H]mannose were obtained from New England Nuclear (Boston, MA). Lithium 3,5-diiodosalicylate was purchased from Eastman Kodak Co. (Rochester, NY). The oligosaccharides, $(AcNeu)_{0-2} \rightarrow [Gal \rightarrow GalNAcOH]$, were isolated from fetuin by treatment with alkaline borohydride as described [8].

Methods

Column chromatography. Sepharose CL-6B columns were equilibrated and eluted with Tris-HCl buffer, pH 8.0 containing 0.1% Triton X-100 and 0.1 mM toluenesulfonyl fluoride; Sephadex G-50, and Bio-Gel P-2 and P-6 columns were equilibrated and eluted with 0.1 M pyridine acetate, pH 5.0. DEAE-Sepharose CL-6B columns were packed according to the manufacturer's instructions and regenerated to the chloride form. After application of the sample, the columns were eluted with linear gradients of LiCl in Tris-HCl buffer, pH 8.0, containing 0.1% Triton X-100 and 0.1 mM toluenesulfonyl fluoride. Affinity chromatography on a WGA-Sepharose column, containing about 5 mg lectin per ml Sepharose, was done as described [7]. The recovery of the radioactivity from the columns was better than 80%.

Digestions with enzymes. Pronase digestion was performed for 96 h at 37°C in 50 mM Tris-HCl buffer, pH 8.0 containing 10 mM Ca^{2+} in the presence of toluene. Pronase dissolved in buffer was added at 0, 24, 48 and 72 h. The enzyme preparation used was free of contaminating exoglycosidases and endo-*N*-acetylhexosaminidases when assayed with *p*-nitrophenyl glycosides and [^{3}H]acetylovalbumin glycopeptides, respectively [6]. Treatment with endo-α-*N*-acetylgalactosaminidase from *D. pneumoniae* was done in 50 mM Tris-maleate buffer, pH 7.6 at 37°C for 24 h in a total volume of 50 μl. *V. cholerae* Neuraminidase digestion was done in 0.1 M sodium acetate buffer, pH 5.6, containing 1 mM $CaCl_2$ using 0.5 unit of enzyme, at 37°C for 24 h. All digestions were terminated by heating at 100°C for 2–5 min.

Alkaline borohydride treatment of the glycopeptides was done with 1.0 M $NaBH_4$ in 0.1 M NaOH for 72–96 h at 37°C under nitrogen in sealed tubes in the dark. The reaction mixture was cooled in an ice bath, the excess borohydride destroyed and the mixture neutralized by careful addition of 1 M acetic acid.

All dialysis and concentrations were carried out at 4°C in Amicon Ultrafiltration units using PM-10 membranes.

Liquid scintillation counting was performed on an Intertechnique Model SL-36 or Model SL-4000 spectrometer equipped with dpm calculating modules. Usually 0.3- or 1.0-ml aqueous samples were mixed with 3 or 10 ml of counting liquid in mini- or regular plastic vials, respectively. Using the external standard method, quenching was not detectable with any of the buffers. Radioactivity on paper chromatogram strips was estimated by extracting cut pieces with 1 ml water in counting vials prior to addition of counting liquid.

Cell cultures. The conditions of cell culture and of labeling of the complex saccharides produced by the cells have been described [1,5].

Preparation of plasma membranes. The cultured cells were treated with 0.02% EGTA in NaCl/P_i and washed twice with 0.15 M NaCl. The cells were suspended in 2 mM $ZnCl_2$, at $2 \cdot 10^8$ cells per 10 ml, and after 15 min at 20°C the suspension was placed in an ice bath [9]. After 5 min, the cells were homogenized in a tight fitting (Type B) Dounce homogenizer until greater than 90% of the cells were broken. The homogenate was centrifuged at $220 \times g$ for 15 min and the pellet was washed once with 10 ml 2 mM $ZnCl_2$.

The membranes were isolated from this pellet by partition in an aqueous two phase polymer system as described by Brunette and Till [10]. Essentially, the pellet was suspended in 10 ml of the top phase, mixed with an equal volume of the bottom phase and centrifuged at $9\,000 \times g$ for 10 min. The supernatant containing the membranes at the interface was transferred to another tube, mixed and re-centrifuged. This process was repeated two or three times. The pellets were combined and used for the isolation of nuclei. The results of the investigation of the nuclear glycoconjugates will be reported elsewhere (Bhava-

nandan, V.P., unpublished data). The material at the interface after the final centrifugation in the two phase system was recovered and subjected to discontinuous sucrose-density centrifugation [11]. The membrane fraction banding between 37% and 41% sucrose was used in our studies.

Solubilization of plasma membrane glycoconjugates. The membranes were delipidated by two extractions with chloroform/methanol (2 : 1, v/v) and then suspended in 5 ml of 0.6 M lithium diiodosalicylate and 0.5% Triton X-100 in 0.1 M Tris-HCl buffer, pH 7.5 [12]. The suspension was stirred at 4°C for 2 h and then centrifuged at 11 000 × *g* for 20 min. This process of extraction and centrifugation was repeated two more times, with the final extraction for 4 h. The combined supernatants, containing 90% and 95% of the ^{3}H- and ^{35}S-labeled components respectively, were dialysed against 50 mM Tris-HCl buffer, containing 0.1% Triton and 0.1 mM toluenesulfonyl fluoride and concentrated.

Marker enzyme assays. 5′-Nucleotidase was assayed by the method of Dewald and Touster [13] and succinate dehydrogenase as described by Pennington [14]. (Na^+, K^+)-activated ATPase and NADPH-diaphorase were determined by the procedures of Wallach and Kamat [15]. Protein was determined according to Lowry et al. [16] with crystalline bovine serum albumin as standard. For electron microscopic examination the membrane fractions were fixed in 2% glutaraldehyde and post fixed with osmium tetroxide. Sections of the embedded material were examined in an RCA model EMU-4 electron microscope. We are indebted to Mr. Barry Hillman for his assistance in this work.

Results

Plasma membranes were obtained in good yields by homogenization of cells in the presence of Zn ions followed by centrifugation in a two phase aqueous polymer system and sucrose density centrifugation. The purity of the membrane preparation was established by assay of marker enzymes and by electron microscopic examination. The membranes obtained from cells metabolically labeled with [^{3}H]glucosamine and $Na_2^{35}SO_4$ were treated with buffer con-

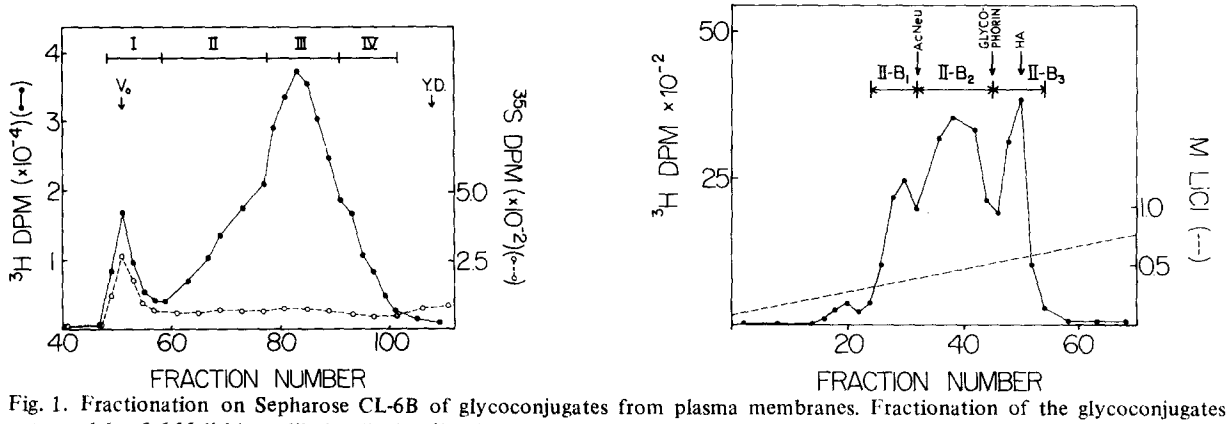
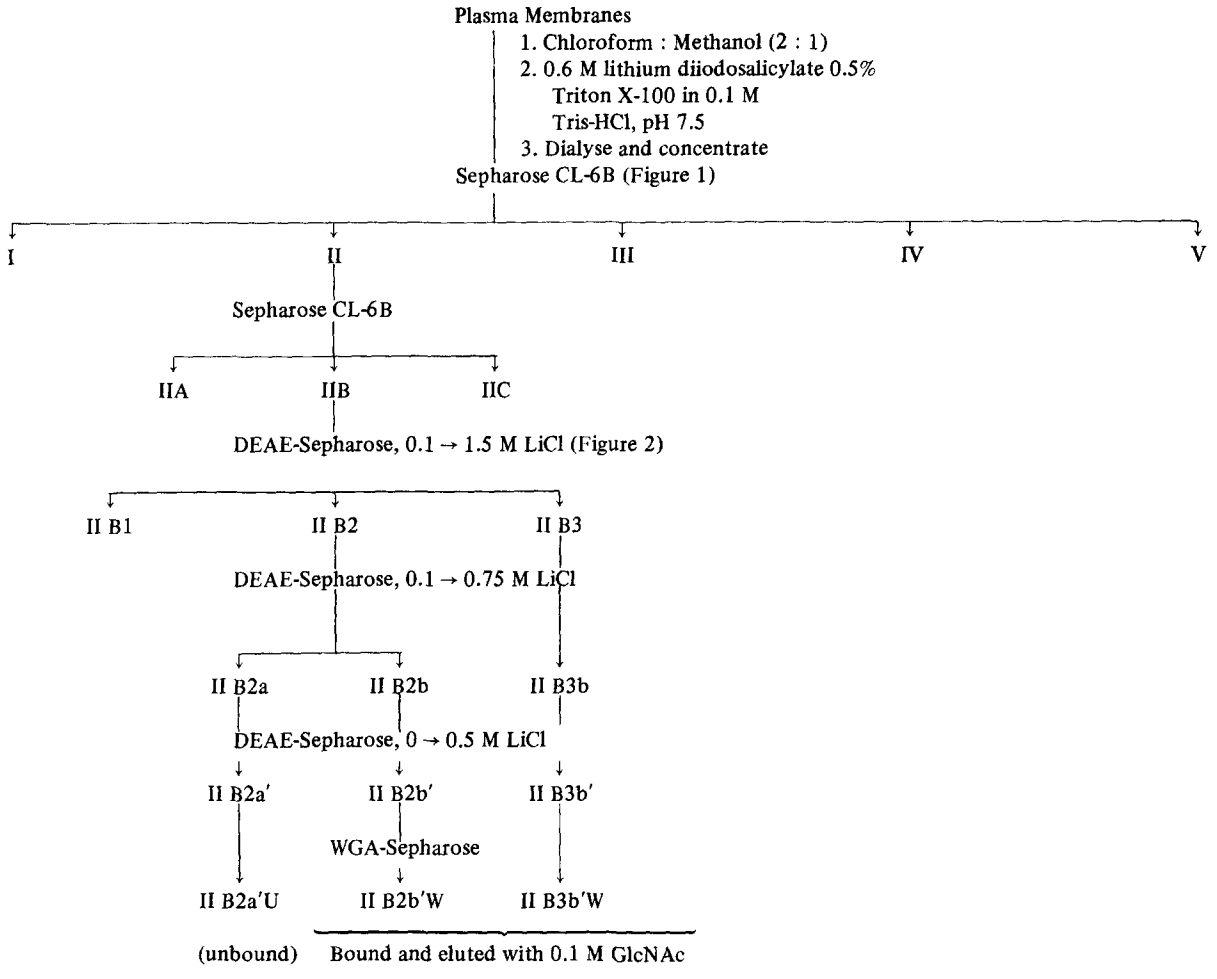


Fig. 1. Fractionation on Sepharose CL-6B of glycoconjugates from plasma membranes. Fractionation of the glycoconjugates extracted by 0.6 M lithium diiodosalicylate/0.5% Triton X-100 from plasma membranes of HM7 human melanoma cells metabolically labelled with [^{3}H]glucosamine and $Na_2^{35}SO_4$. The extracts were dialysed against 50 mM Tris-HCl buffer, pH 8.0, containing 0.1% Triton X-100 and 0.1 mM toluenesulfonyl fluoride, concentrated and chromatographed in the same buffer on a column of Sepharose CL-6B (1.5 × 100 cm). Fractions (1.2 ml) were collected and aliquots analyzed for radioactivity. Fractions were combined as indicated and concentrated to yield the numbered peaks. Peak elution positions of blue dextran (V_0) and yellow dextran (Y.D.) are indicated by arrows.

Fig. 2. Fractionation of glycoprotein fraction IIB on DEAE-Sepharose. The glycoprotein Fraction IIB from the second gel filtration on Sepharose CL-6B was chromatographed on a DEAE-Sepharose column (1.5 × 18 cm). The column was eluted with a linear gradient of 0.1 to 1.5 M LiCl in 50 MM Tris-HCl, pH 8.0, containing 0.1% Triton X-100 and 0.1 mM toluenesulfonyl fluoride. Fractions of 1 ml were collected and aliquots analyzed for radioactivity. The three fractions indicated by horizontal arrows were recovered by dialysis and concentration. The peak elution positions of AcNeu, glycophorin and hyaluronic acid (HA) are indicated.



Scheme 1: Isolation of Glycoproteins from Plasma Membranes of HM7 Human Melanoma Cells.

taining various detergents, singly or in combinations [17]. The combination of 0.6 M lithium diiodosalicylate and 0.5% Triton was found to be the best since it solubilized about 90 and 95% of the ^{3}H- and ^{35}S-labeled components, respectively.

Fractionation of the solubilized glycoconjugates

The fractionation of the solubilized plasma membrane glycoconjugates on a column of Sepharose CL-6B is illustrated in Fig. 1. The five peaks obtained were designated I, II, III, etc. The material from each peak was digested with pronase and the resultant glycopeptides fractionated by gel filtration on a Sephadex G-50 column into peak A (excluded) and peaks B and C (included). Hexosamine analysis, affinity chromatography on WGA-Sepharose and treatment with alkaline borohydride of the three glycopeptide peaks revealed that peak A was enriched in the Class I (mucin-type) glycopeptides reported previously [1]. The Sepharose CL-6B fractions I and II were both rich in the peak A glycopeptides but fraction II, containing 22% of the total ^{3}H-radioactivity, was chosen for further purification since it was low in $^{35}SO_4$ and thus in sulfated glycosaminoglycans. Rechromatography of II on Sepharose CL-6B gave a rather broad peak and the material in fractions 60–77 was recovered to yield IIB. Chromatography of IIB on a DEAE-Sepharose column using a 0.1 → 1.5 M LiCl gradient (Fig. 2) resulted in three peaks (IIB1, IIB2 and IIB3). Fractions IIB2 and IIB3 were sepa-

rately rechromatographed on the DEAE-Sepharose column but using a gradient of 0.1 → 0.75 M LiCl for elution, to yield three fractions IIB2a, IIB2b and IIB3b. Each of these fractions was chromatographed once more on DEAE-Sepharose columns using 0 → 0.5 M LiCl gradients and the material eluting in the sharp symmetrical areas of the peaks recovered. Additional purification of the peak materials was achieved by affinity chromatography on a WGA-Sepharose column. The major portion (84%) of IIB2a′ which passed through the column was recovered and designated IIB2a′ U. In the case of IIB2b′ and IIB3b′, 88 and 99%, respectively, bound to the WGA-Sepharose and could be eluted with 0.1 M *n*-acetylglucosamine. The materials recovered by dialysis and concentration were designated IIB2b′W and IIB3b′W. The three purified glycoproteins contained about 7% of the total ^{3}H-radioactivity in the glycoconjugates (lithium diiodosalicylate extract); see Scheme I.

Characterization of the glycoprotein fractions

Polyacrylamide gel electrophoresis [18] of the three purified glycoproteins in 6% gel in the presence of sodium dodecyl sulfate gave single bands. Gel filtration of the glycoproteins on a Sepharose CL-6B column using buffer containing 0.1% Triton X-100 gave single homogenous peaks in each case. The apparent molecular weight of IIB3b′W was estimated to be about 100 000 based on gel filtration on a column calibrated with glycoprotein standards (Fig. 3).

TABLE I

DISTRIBUTION OF TRITIUM ACTIVITY IN SIALIC ACID AND HEXOSAMINES OF PURIFIED GLYCOPROTEINS

The proportions of sialic acid in three purified glycoproteins were determined by hydrolysis (0.1 N H_2SO_4, 80°C, 1 h), neutralization and gel filtration on a BioGel P-2 column. The tritium activity co-eluting with [^{14}C]AcNeu internal standard was estimated. Hexosamine was determined on acid hydrolysates (4N HCl, 100°C, 8 h) on an amino acid analyser by a streamsplitting technique (22).

Glycoprotein	Sialic Acid (%)	$GlcNH_2$ (%)	$GalNH_2$ (%)
II B2a′U	20.0	78.8	1.2
II B2b′W	26.3	49.9	23.8
II B3b′W	37.1	1.9	61.0

The distribution of tritium activity in sialic acid and hexosamines in the three glycoproteins is given in Table I.

Elucidation of the nature of oligosaccharides in IIB3b′W

The glycoprotein was subjected to alkaline borohydride treatment and chromatographed on a calibrated BioGel P-6 column. The ^{3}H-labeled carbohydrate was almost completely (94%) eliminated and about 55 and 29% eluted in the positions of the tetrasaccharide [AcNeu → Gal → (AcNeu) → GalNAc-(OH)] and the trisaccharide [AcNeu → Gal → GalNac-(OH)], respectively (Fig. 4). When the material from these two peaks were isolated, treated with *V. cholerae* neuraminidase and rechromatographed on the

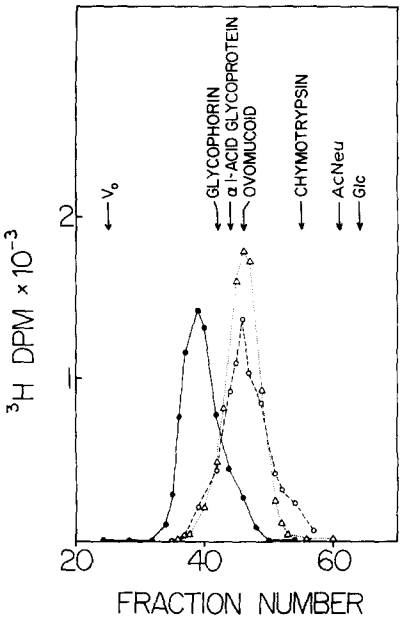


Fig. 3. Gel filtration of IIB3b′W on Sepharose CL-6B before and after pronase digestion. The glycoprotein IIB3b′W before (•——•) or after (○- - - - -○) pronase digestion was mixed with [^{14}C]glucose and chromatographed on a Sepharose CL-6B column (1.5 X 35 cm). The column was eluted with 50 mM Tris-HCl, pH 8.0, containing 0.1% Triton X-100 and 0.1 mM toluenesulfonyl fluoride. 1-ml fractions were collected and analyzed for radioactivity. Human melanoma Class I glycopeptide GPIb (△ · · · · · · △) was also similarly chromatographed. The results are presented by superimposing the elution patterns using [^{14}C]glucose as an internal reference. The peak elution positions of blue dextran (V_0), AcNeu, Glc and several glycoproteins are indicated by arrows.

same column the radioactivity eluted in a single peak coincident with Gal → GalNAc(OH) and AcNeu (Fig. 4). The presence of Gal → [^{3}H]GalNAc(OH) and AcNeu in this peak, as well as the identity of the tetra- and trisaccharides were confirmed by paper chromatography in two solvent systems; *n*-butyl-acetate/glacial acetic acid/water (3 : 2 : 1, v/v) and pyridine/ethyl acetate/water/glacial acetic acid (5 : 5 : 3 : 1, v/v) [1].

The nature of the carbohydrate moieties of this glycoprotein (IIB3b'W) was further explored by treating the asialoglycoprotein (prepared by mild acid hydrolysis) with endo-α-*N*-acetylgalactosaminidase. The released product, consisting of 87% of the radioactivity in the asialocompound, co-eluted on a Bio-Gel P-2 column with Gal(1 → 3)GalNAc prepared by treatment of asialofetuin glycopeptide with the same enzyme [19]. The radioactive product was isolated, desalted by passage through mixed bed ion exchange and examined by paper chromatography

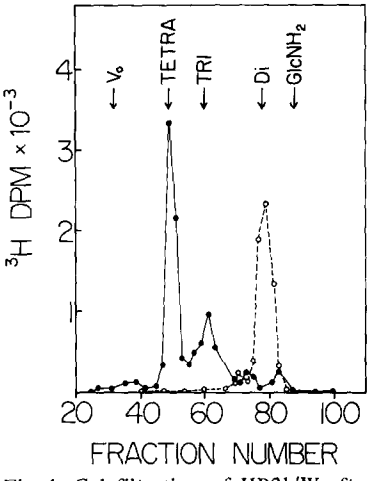


Fig. 4. Gel filtration of IIB3b'W after alkaline borohydride treatment. The glycoprotein IIB3b'W was treated with alkaline borohydride (•———•) and chromatographed on a BioGel P-6 column (0.9 × 90 cm). The column was eluted with 0.1 M pyridine/0.1 M acetic acid, pH 5.0, 1-ml fractions collected and aliquots analyzed for radioactivity. The materials eluting in fractions 47–53 and 57–67 were recovered by lyophilization and portions examined by paper chromatography. Separate portions were treated with *Vibrio cholerae* neuraminidase and re-chromatographed on the same column. In both cases, the radioactivity eluting in single peaks (o- - - - -o) was recovered by lyophilization. The peak elution positions of blue dextran (V_0) and reference saccharides are indicated by arrows.

using *n*-butylacetate/glacial acetic acid/water (3 : 2 : 1, v/v) as solvent [20]. The results confirmed the identity of the product as Gal(1 → 3)[^{3}H]GalNAc.

Isolation and characterization of glycopeptides from IIB3b'W

The glycoprotein was treated with pronase, centrifuged at 1 000 × *g* for 10 min and the glycopeptides in the supernatant examined by gel filtration on Sephadex G-50 and Sepharose CL-6B columns. On the Sephadex G-50 column, about 90% of the labeled material eluted at the void volume, indicating the presence of mostly peak A glycopeptide mentioned previously. On a Sepharose CL-6B column the material was included and eluted in the same area as the peak A glycopeptides derived from the crude glycoprotein (Fig. 3). The glycopeptide purified by chromatography on Sepharose CL-6B had no detectable [^{3}H]GlcNH$_2$; the tritium label was entirely in AcNeu and GalNH$_2$. Treatment of the glycopeptide with alkaline borohydride gave a mixture of AcNeu → Gal → (AcNeu) → GalNAc(OH) and AcNeu → Gal → GalNAc(OH). The glycopeptide was estimated to have an apparent molecular weight in the range of 12 000–15 000 by gel filtration and showed sialic acid-dependent interaction with WGA-Sepharose [7]. Redigestion of the glycopeptide with pronase did not alter its chromatographic elution profiles or its interaction with WGA.

[^{3}H]Mannose labeling of the HM7 glycoproteins

Plasma membranes were isolated from cells grown in the presence of [^{3}H]mannose and [^{14}C]glucosamine (5 μCi and 1 μCi per ml culture medium). The labeled glycoconjugates were extracted and fractionated by repeated chromatography on Sepharose CL-6B and DEAE-Sepharose columns as described above. The most acidic glycoprotein IIB3b'W, had negligible incorporation of label from [^{3}H]mannose confirming the absence of *N*-glycosidically-linked oligosaccharides. The two other glycoproteins purified, IIB2a'U and IIB2b'W, contained significant quantities of tritium radioactivity indicating the presence of *N*-glycosidically-linked oligosaccharides.

Examination of FM13 human fetal uveal melanocytes for mucin-type glycoproteins

The glycoconjugates were isolated by extraction of

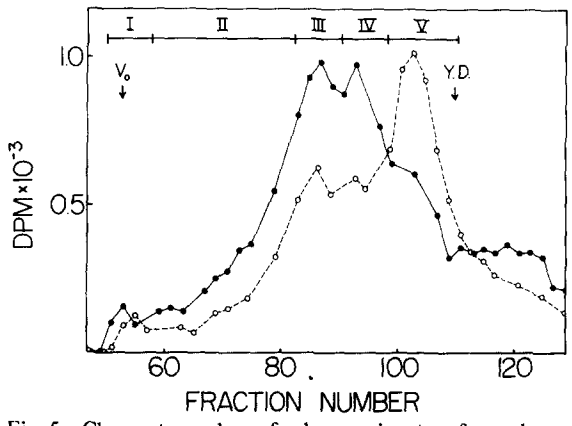


Fig. 5. Chromatography of glycoconjugates from human melanoma cells and human fetal melanocytes. Glycoconjugates isolated from HM7 human melanoma cells (•——•) and from FM13 human fetal melanocytes (o- - - - -o), metabolically labelled with [^{3}H]glucosamine, were chromatographed on Sepharose CL-6B column (1.5 X 100 cm) as described in Fig. 1. The results are presented by superimposing the elution profiles. The materials eluting in the area indicated by 'II' were recovered and further examined.

whole cells grown in the presence of [^{3}H]glucosamine with 0.6 M lithium diiodosalicylate/0.5% Triton X-100. The extracted glycoconjugates were partially purified by partition in an aqueous three-phase polymer system consisting of polyethylene glycol, dextran and Ficoll [21]. This step removes nucleic acids and separates (glyco)proteins according to hydrophobicity. The ^{3}H-labeled glycoconjugates in the upper phase were recovered after dialysis. The elution profile of these glycoconjugates and of the glycoconjugates similarly prepared from labeled HM7 melanoma cells on a Sepharose CL-6B column is illustrated in Fig. 5. It is clear that the melanoma cells produce an increased proportion of glycoproteins eluting in the higher molecular size region (fractions 61–95) of the column, whereas the bulk of the glycoprotein from the melanocytes eluted in the lower molecular size region of the same column. These results are consistent with our earlier observation that the melanoma cultures produced a higher proportion of the larger glycopeptides than the control cells [1].

The labeled material eluting in the fractions marked 'II' was recovered and chromatographed on a WGA-Sepharose 4B column. In the case of the HM7 Fraction II, 70% of the labeled material bound to the column and was recovered by elution with 0.1 M GlcNAc. In contrast, only 49% of the labeled material of the FM13 Fraction II was bound to and eluted from the WGA-Sepharose. The WGA-interacting glycoproteins were recovered by dialysis and chromatographed on a DEAE-Sepharose column (Fig. 6). The elution of the purified ^{14}C-labeled glycoprotein (IIB3b'W) isolated from plasma membranes of HM7 cells on the same column is also illustrated in Fig. 6.

A portion of the FM13 Fraction II (glycoproteins) was digested with pronase and the glycopeptides examined on a Sephadex G-50 column. Only 8.2% of the labeled material eluted at the void volume (peak A glycopeptide), the balance was included. This is in contrast to the elution of about 28.6% in peak A when HM7 Fraction II material was subjected to the same treatment.

Discussion

Our major aim in these studies was the isolation and characterization of glycoprotein(s) rich in carbohydrate groups linked via *O*-glycosidic linkage to proteins. Accordingly, the five fractions obtained by chromatography on Sepharose CL-6B (Fig. 1)

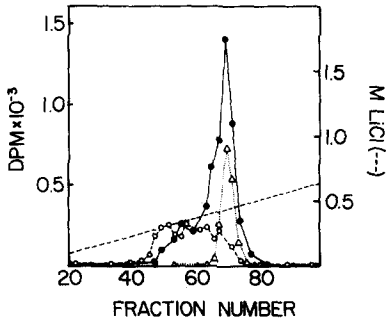


Fig. 6. Chromatography of melanoma and melanocyte glycoproteins (fraction II) on DEAE-Sepharose. The fraction II glycoproteins from melanoma cells and melanocytes (Fig. 5) were purified by affinity chromatography on WGA-Sepharose. The WGA-interacting glycoproteins from melanoma (•——•) and melanocyte (o- - - - -o) were each mixed with ^{14}C-labeled glycoprotein IIB3b'W (△· · · · ·△) and chromatographed on DEAE-Sepharose column (1.5 X 18 cm). The column was eluted with a linear gradient of 0.1 to 1.0 M LiCl in 50 mM Tris-HCl, pH 8.0, containing 0.1% Triton X-100 and 0.1 mM toluenesulfonyl fluoride. Fractions of 1 ml were collected and analyzed for radioactivity. The results are presented by superimposing the elution patterns.

were examined for the presence of WGA-binding glycopeptides (GPIa and GPIb) previously described [1].

Purification of fraction II using buffers containing 0.1% Triton X-100 and 0.1 mM toluenesulfonyl fluoride yielded three apparently homogenous glycoproteins. The recovery of the glycoproteins was poor in the absence of detergents, apparently due to aggregation and precipitation [22]. The molecular weight of the most acidic glycoprotein (IIB3b'W) was estimated as 100 000 by gel filtration [22]. The gel electrophoresis data indicate a smaller subunit molecular weight, but the anomalous behavior of glycoproteins in the presence of dodecyl sulfate [23] does not permit an accurate estimation by this technique.

The sialic acid and hexosamine analysis indicated that the glycoprotein which had the highest affinity for DEAE-Sepharose (IIB3b'W) had the highest sialic acid and GalNAc contents, whereas the glycoprotein (IIb2a'U) with the least affinity had the lowest sialic acid and highest GlcNAc content. The low sialic acid content of this glycoprotein is also consistent with its noninteraction with WGA-Sepharose [7]. The isolated glycoproteins may be considered as representative of three general classes of glycoproteins, containing only *N*-glycosidically linked or only *O*-glycosidically linked or both types of oligosaccharides [24]. These macromolecules did not contain detectable ^{35}S radioactivity and therefore, are not hybrid glycoprotein-glycosaminoglycan molecules of the type described by Baker et al. [25].

The high galactosamine content of IIB3b'W and the lack of mannose suggested that this glycoprotein had a mucin-type carbohydrate structure. The *O*-glycosidically-linked oligosaccharide was shown to be mainly tetra- and trisaccharides of the type $(AcNeu)_{1\ or\ 2} \rightarrow [Gal \rightarrow GalNac(OH)]$.

To the best of our knowledge, an intrinsic membrane glycoprotein having carbohydrate entirely *O*-glycosidically linked to the protein has not been previously described. Several membrane glycoproteins rich in mucin-type oligosaccharides but also having serum-type oligosaccharides are known. These include glycophorin (M_r 31 000) from human erythrocyte ghost [26,27], a glycoprotein (M_r about 100 000) from plasma membranes of ascites hepatoma AH 66 [12,28], bovine [29] and porcine [30] erythrocyte membrane and thyroid plasma membrane [31] glycoproteins. An extrinsic mucin-type glycoprotein (epiglycanin) has been isolated from TA3 Ha mouse mammary carcinoma cells and extensively investigated by Codington and co-workers [32,33]. In preliminary experiments, the human melanoma glycoprotein IIB3b'W failed to complex with rabbit anti-human glycophorin antibodies (Bhavanandan, V.P., unpublished data).

The FM13 melanocytes produced significantly lesser proportions of the WGA-binding glycoproteins identical or similar to IIB3b'W (Fig. 6). This is in agreement with our previous results that pronase digests of cultured human fetal uveal melanocytes or spent culture medium yielded markedly reduced quantities of the WGA-binding mucin-type glycopeptides GPIa and GPIb [1].

In future investigations we hope to isolate mg quantities of the described glycoproteins by extraction of membranes prepared from HM7 tumors grown in athymic mice for additional biochemical and immunological characterizations.

Acknowledgements

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