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IDENTIFICATION OF EXPOSED SURFACE GLYCOPROTEINS IN UNDIFFERENTIATED AND DIFFERENTIATED MOUSE N-18 NEUROBLASTOMA CELLS

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A simple method is described that permitted rapid isolation of plasma membranes from mouse N-18 neuroblastoma cells. The purified plasma membranes gave a 10-fold increase in the specific activity of incorporated [^{3}H]fucose over that of the cell homogenate. The specific activities of two other membrane markers, 5′-nucleotidase and alkaline phosphatase, increased 11-fold and 15-fold, respectively. Metabolic labeling with [^{3}H]fucose identified a major fucosyl glycoprotein with apparent molecular weight of 92000. Three surface labeling methods together with SDS-polyacrylamide gel electrophoresis and fluorography were used to characterize and compare the surface glycoproteins of undifferentiated and differentiated N-18 cells. The galactose oxidase/NaB^{3}H$_4$ method labeled two major galactoproteins (M_r = 52000, 42000) in both undifferentiated and differentiated cells. The neuraminidase/galactose oxidase/NaB^{3}H$_4$ method revealed many sialylgalactoproteins. Among them, the 220-kdalton, 150-kdalton and 130-kdalton bands were at least 100% more prominently labeled in the differentiated calls whereas the 76-kdalton and 72-kdalton bands were less prominently labeled in the differentiated cells when compared to their undifferentiated counterparts. The prominently iodinated protein bands in the undifferentiated cells had apparent molecular weights of 130000, 92000, 76000 and 72000 as compared to 150-, 130-, 92- and 76-kdalton bands in the differentiated cells. The labeling data obtained will enable us to further study the changes of these identified surface glycoproteins, both quantitatively and topologically, during the differentiation of neuroblastoma cells.

Introduction

Mouse neuroblastoma cells in tissue culture can be induced to differentiate by the addition of cyclic AMP analogs and agents which increase intracellular cyclic AMP concentration [1–3]. The differentiation of neuroblastoma cells is characterized by morphological appearance of long neurite outgrown (> 50 μm) and increased activities of enzymes involved in neurotransmitter metabolism [1–3]. In addition, the differentiated neuroblastoma cells lose their tumorigenicity [4].

The importance of plasma membranes in various growth regulatory processes is well documented [5–7]. The precise role played by each individual component of plasma membranes and the molecular mechanism(s) involved in a particular growth regulatory process, however, remain to be elucidated.

Detailed information on the surface-exposed

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Abbreviations: dibutyryl cyclic AMP, N^6,$O^{2'}$-dibutyryl adenosine 3′:5′-cyclic monophosphate; IBMX, 1-methyl-3-isobutylxanthine; PMSF, phenylmethylsulfonyl fluoride; SDS, sodium dodecyl sulfate; Hepes, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid.

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groups of cells is necessary for understanding various phases of membrane-mediated processes. In this regard, mouse neuroblastoma cells in tissue culture constitute a good system to investigate possible involvement of surface proteins in the differentiation/tumor reversion process [1–4]. Due to their neuronal origin, mouse neuroblastoma cells also provide a useful model to study the pattern of expression of various membrane properties associated with neuronal functions such as synaptic formation and excitability [8,9].

Surface glycoproteins are important components of plasma membranes, and together with glycolipids, account for the membrane's carbohydrate content [5,6]. So far, only metabolic labeling methods using [^{3}H]fucose or [^{3}H]glucosamine have been employed to study surface glycoproteins of mouse neuroblastoma cells. For example, Trudding et al. [10] showed an increased incorporation of radioactive fucose and glucosamine into a 105-kdalton protein in differentiated mouse N2a neuroblastoma cells whereas Littauer et al. [11] showed a preferential incorporation of [^{3}H]fucose into a 200-kdalton protein of differentiated N-18 neuroblastoma cells. On the other hand, Haffke and Seeds [12] found no major difference in the labeling patterns between undifferentiated and differentiated cells using either [^{3}H]fucose or [^{3}H]glucosamine as precursor. Since metabolic labeling depends on the metabolic activity of the cells under investigation, the difference in the labeling patterns observed between the undifferentiated and differentiated neuroblastoma cells may simply reflect the difference in the biosynthesis/biodegradation of fucose-containing or glucosamine-containing glycoproteins and do not necessarily represent true difference of the in situ composition of these glycoproteins.

In order to compare the composition of surface proteins of two different cell populations (such as undifferentiated versus differentiated neuroblastoma cells), other surface labeling methods using chemical or enzyme catalyzed reactions are more desirable.

In the present study, we used metabolic labeling for developing a membrane isolation procedure for both undifferentiated and differentiated N-18 neuroblastoma cells. The composition of exposed surface glycoproteins in the undifferentiated and differentiated N-18 cells was probed and compared, however, by galactose oxidase-reductive tritiation (galactose oxidase/NaB^3H_4) method and neuraminidase-galactose oxidase-catalyzed reductive tritiation (neuraminidase/galactose oxidase/NaB^3H_4) method, which characterize galactosyl/*N*-acetylgalactosaminyl glycoproteins and sialylgalactosyl/sialyl-*N*-acetylgalactosaminyl glycoproteins, respectively. In addition, the lactoperoxidase-catalyzed iodination (lactoperoxidase/^{125}I) method was also used for comparative purpose.

Material and Methods

L-[^{3}H]Fucose (0.95 Ci/mmol), NaB^3H_4 (10 Ci/mmol) and $Na^{125}I$ were purchased from Amersham, Arlington Heights, IL. Dulbecco's modified Eagle medium, Earle's balanced salt solution and fetal calf serum were obtained from Gibco, Grand Island, NY. $N^6,O^{2'}$-Dibutyryl adenosine 3′:5′-cyclic monophosphate (dibutyryl cyclic AMP), phenylmethylsulfonyl fluoride (PMSF), D-galactose oxidase (EC 1.1.3.9), D-glucose oxidase (EC 1.1.3.4) and lactoperoxidase (EC 1.11.1.7) were obtained from Sigma Chemical Co., St. Louis, MO. Neuraminidase (500 units/ml or 1 I.U./ml) was supplied from Calbiochem-Behring Corp., San Diego, CA. 1-Methyl-3-isobutylxanthine (IBMX) was purchased from Aldrich Chem. Co., Milwaukee, WI. All other chemicals were of reagent grade. We wish to thank Dr. E.S. Canellakis of the Department of Pharmacology, Yale Medical School, for providing us with mouse N-18 neuroblastoma cells.

Cell culture and differentiation. Mouse N-18 neuroblastoma cells were grown as monolayer cultures in Dulbecco's modified Eagle medium (with 4500 mg glucose per liter, without sodium pyruvate) supplemented with 10% fetal calf serum. Cells were maintained at 37°C in a Forma water-jacketed CO_2 incubator (95% air/5% CO_2). In this study, differentiation, defined as the morphological appearance of neurites > 50 μm in length and the biochemical expression of increased acetylcholinesterase activity, was induced by adding dibutyryl cyclic AMP (1 mM) and IBMX (0.5 mM) to the sparse culture 15 h after subculture. Both undifferentiated and differentiated neuroblastoma

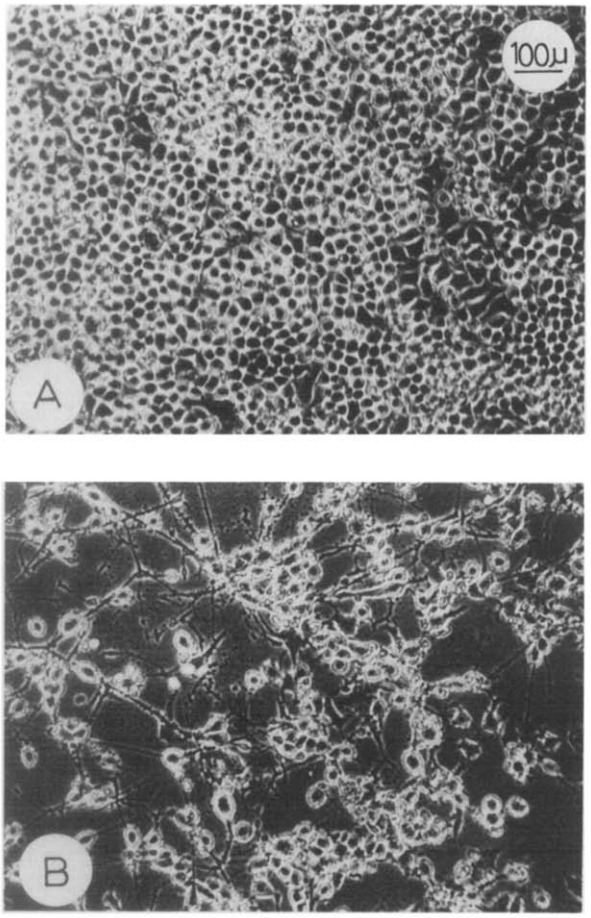

Fig. 1. Photomitograph of stationary-phase NB (A) and ND (B) cells.

cells at their early stationary phase of growth were used for all the following experiments, they were designated as NB and ND cells respectively. Fig. 1 is the photomicrograph of NB and ND cells. Two features can be noted: (a) at stationary phase of growth, NB cells were confluent and with saturation density approx. 4 to 6 times that of ND cells; (b) ND cells were characterized by extensive webs of long neurites.

Galactose oxidase catalyzed reductive tritiation. We used the procedure of Gahmberg and Hakomori [13] with some modifications. Briefly, cells were harvested and resuspended in Earle's balanced salt solution at a concentration of approximately $5 \cdot 10^7$ cells/ml. Galactose oxidase (10 units/ml) was added to the cell suspension and incubated at 35°C for 40 min with occasional gentle shaking. Cells were then washed twice with phosphate-buffered saline and resuspended in a small volume of 0.106 M phosphate buffer (pH 7.2) (approx. $2 \cdot 10^8$ cells/ml). Reductive tritiation was initiated by adding an aliquot of NaB^3H_4 solution to the cell suspension (final concentration of NaB^3H_4 was 2 mCi/ml). The reaction was carried out at room temperature for 30 min and was terminated by diluting the cell suspension with 20 volumes of cold phosphate-buffered saline. To radiolabel the sialylgalactosyl/sialyl-*N*-acetylgalactosaminyl glycoproteins, cells were pretreated with neuraminidase as previously described [13]. Alternatively cells were treated with both neuraminidase and galactose oxidase simultaneously for 40 min at 35°C. We found no significant difference in the labeling pattern obtained by either treatment.

Metabolic labeling. Fifteen to twenty hours before cells reached stationary phase of growth, L-[^{3}H]fucose was added to the cell culture (1 μCi/ml) under sterile conditions.

Lactoperoxidase catalyzed iodination. We used the same procedure as previously described by Hubbard and Cohn [14]. Briefly, cells were rinsed and suspended in Earle's balanced salt solution (cell concentration was about $5 \cdot 10^7$ cells/ml). To this cell suspension 50 μg/ml lactoperoxidase, 0.1 mCi/ml carrier-free ^{125}I, 20 mM glucose and 50 mU/ml glucose oxidase were added. The reaction was carried out at 4°C for 20 min.

Isolation of plasma membrane. Plasma membrane fractions from NB and ND cells were isolated by hypotonic disruption and two step discontinuous sucrose gradient centrifugation [15]. Briefly, cells were rinsed twice with cold phosphate-buffered saline and removed from the substratum by flushing with a stream of cold phosphate-buffered saline, cells were then sedimented by centrifuging at $1000 \times g$ for 2 min. The cell pellet was resuspended in a homogenizing medium containing 1 mM $MgCl_2$, 2 mM $CaCl_2$ and 0.5 mM PMSF in 10 mM Hepes (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) buffer (pH 7.2) at a concentration of approx. $2 \cdot 10^7$ cells/ml. Cells were ruptured by forcing 3-ml portions of the cell suspension through a 5-ml BD plastic syringe fitted with 25 gauge/5/8 inch

needle. From three to six strokes were needed to disrupt the cells as monitored by phase contrast microscopy in the presence of Trypan blue dye. More than 90% of the nuclei remained intact. The homogenate was centrifuged at 1300 × *g* for 2 min; the pellet was suspended in 4 volumes of homogenizing medium and centrifuged again at 1300 × *g* for 10 min; the combined supernatants were brought to 10% sucrose by adding a 60% sucrose solution and the pool was designated 'the supernatant fraction'. From 3 to 5 ml of the supernatant fraction was then applied on a discontinuous sucrose gadient (7 ml 50% sucrose, 14 ml 30% sucrose) and centrifuged at 23300 × *g* for 30 min in a Sorvall RC-5 centrifuge using the HB-4 rotor. The 10%–30% interface layer was collected, diluted with phosphate-buffered saline containing 1 mM PMSF, and sedimented by centrifuging at 33000 × *g* for 30 min. The pellet was resuspended in a small volume of saline (0.9%), and designated 'the I-30 fraction'. The I-30 fraction was applied on a second discontinuous gradient (3 ml 50%, 5 ml 30% and 3 ml 15% sucrose) and centrifuged for 30 min at 23300 × *g*. The 15%–30% interface was collected, diluted with phosphate-buffered saline containing 1 mM PMSF and sedimented by centrifugation. The pellet was resuspended in a small volume of saline and designated 'the II-30 fraction'. Based on the increase of specific activities of 5′-nucleotidase, alkaline phosphatase and radioactivity of incorporated L-[^{3}H]fucose (see Table I), we designated II-30 'plasma membrane fraction'. The enzyme activities and the incorporation of L-[^{3}H]fucose into acid-insoluble material were assayed by methods previously described [15]. Protein concentrations were determined by the procedure of Lowry et al. [16].

Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis Protein sample containing 0.5–3.0 mg/ml protein was mixed with one-fifth of its volume of SDS-stop solution containing 12% SDS, 0.5 M tris-HCl (pH 9.0), 10% β-mercaptoethanol, 5 mM EDTA, 25% glycerol and 0.005% of tracking dye Pyronin Y and heated at 100°C for 5 min. Samples containing 50 μg of protein were applied on a linear gradient slab gel (7.5%–15% acrylamide) as previously described [15].

Autoradiograms and fluorograms were made on Kodak X-ray film as previously described [15]. For the densitometric tracing, fluorograms or autoradiograms were scanned with a Schoeffel SD-3000 spectromicrodensitometer. Apparent molecular weights of the radioactive bands were estimated by the method of Fairbanks et al. [17]. Standard proteins (and their molecular weights) used were cytochrome *c* (13000), chymotrypsinogen (25000), aldolase (40000), ovalbumin (45000), catalase (58000), bovine serum albumin (67000), phosphorylase *a* (94000) and β-galactosidase (130000).

Results

Purity of plasma membranes

The purity of the isolated plasma membrane fraction was determined by the increases of specific activities of two marker enzymes, 5′-nucleotidase and alkaline phosphatase, and the incorporation of L-[^{3}H]fucose into acid-insoluble material. Table I shows that after two discontinuous sucrose gradient centrifugations, the plasma membrane fraction (II-30) obtained had about a 11- and 15-fold enrichment in 5′-nucleotidase and alkaline phosphatase activities, respectively. These values are comparable to those reported by Garvican and brown [18] for NB41A neuroblastoma cells. Several studies have demonstrated that L-fucose is incorporated very specifically into glycoproteins of the plasma membranes of nucleated eukaryotes [19,20]. In our plasma membrane preparation (II-30 fraction), a 10-fold increase in the specific activity of [^{3}H]fucose over that of the cell homogenate was observed. It should be noted that, however, Garvican and Brown [18] reported a 38-fold increase of specific activity of [^{3}H]fucose in their membrane preparation, a value that is 3-times higher than the enrichment of the marker enzymes. Such discrepancy may be due to the difference in the clonal cell lines used. Fig. 2 shows the densitometric tracings of the electrophoretic patterns of both plasma membrane fraction and cell homogenate obtained from N-18 cells incubated with [^{3}H]fucose for 20 h. This result indicated that the incorporated [^{3}H]fucose in N-18 cells was associated with plasma membranes in the form of fucosylated glycoproteins. As can be seen in Fig. 2, six fucosyl glycoproteins were identifiable with apparent molecular weights of 250000, 220000, 150000, 130000, 92000, 72000 and 46000.

TABLE I

DISTRIBUTION OF PLASMA MEMBRANE ENZYME MARKERS AND INCORPORATED [^{3}H]FUCOSE AMONG SUBCELLULAR FRACTIONS OF N-18 NEUROBLASTOMA CELLS

I-30 and I-50 represent, respectively, the 10%–30% interface fraction and 30%–50% interface fraction obtained after the first discontinuous sucrose gradient centrifugation. II-30 and II-50 represent, respectively, the 15%–30% interface fraction and 30%–50% interface fraction obtained after the second discontinuous sucrose gradient centrifugation.

Fraction	Total protein (mg)	5′-Nucleotidase (μmol/mg/h)	Alkaline phosphatase (μmol/mg/h)	Acid-insoluble radioactivity (cpm/μg)
Homogenate	26.58	0.210	0.120	55
Supernatant	16.30	0.250	0.166	51
I-30	1.55	0.685	0.423	214
I-50	2.58	0.320	0.319	105
II-30	0.1	2.230	1.795	490
II-50	0.53	1.500	0.415	320

Among them, the 92-kdalton protein was the most prominent one and accounted for more than 75% of total incorporated radioactivity.

Cell surface labeling using agalactose oxidase/ NaB^3H_4 method

Galactose oxidase/NaB^3H_4 method has been used successfully in characterizing exposed surface

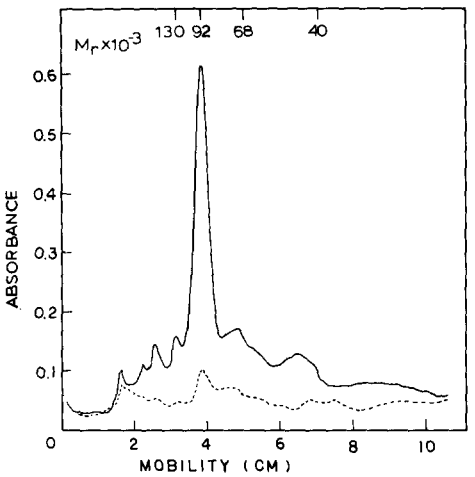

Fig. 2. Densitometric tracings of SDS-polyacrylamide slab gel electrophoresis of fucosylated glycoproteins from plasma membrane fraction (solid line) and cell homogenate (dotted line) of N-18 mouse neuroblastoma cells. Plasma membrane fraction used in this experiment had 9.2-fold increase of alkaline phosphatase activity as compared to that of cell homogenate. Each sample applied to the slab gel contained 50 μg protein.

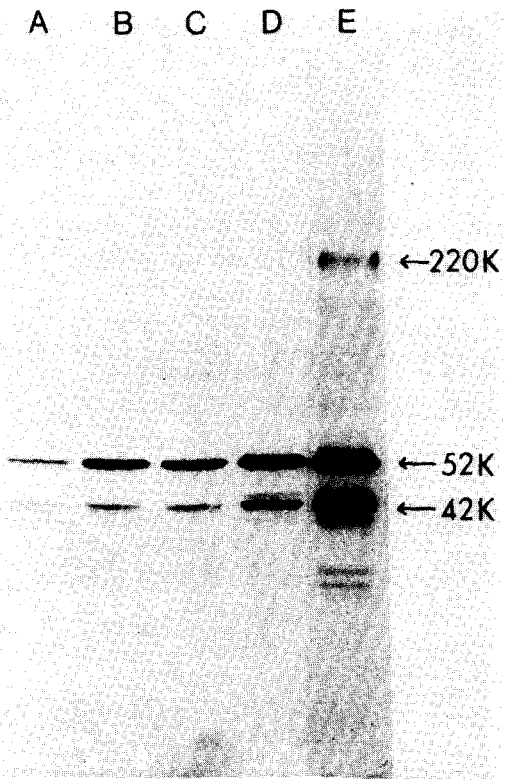

Fig. 3. Fluorograph of SDS-polyacrylamide gel electrophoresis of surface glycoproteins of N-18 neuroblastoma cells labeled by the galactose oxidase/NaB^3H_4 method. Lane A, cell homogenate; lane B, crude plasma membrane fraction (I-30 fraction); lane C, crude membrane fraction (II-50 fraction); lane D, plasma membrane fraction (II-30 fraction); lane E, same as lane D except that the exposure time was increased three times. Each lane contained 50 μg protein.

glycoproteins with D-galactose and/or 2-acetoamido-2-deoxy-D-galactose (*N*-acetylgalactosaminyl) residues at the non-reducing terminal of the carbohydrate moieties [21]. In the present study, this method revealed two major galactosyl/*N*-acetylgalactosaminyl glycoproteins with apparent molecular weights of 52000 and 42000 (Fig. 3). Fig. 3 shows the patterns of various cell fractions obtained after galactose oxidase/NaB^3H_4 treatment. The fact that the same labeling patterns were obtained in the cell homogenate (Fig. 3, Lane A), crude membrane fractions (Fig. 3, Lanes B and C) and plasma membrane fraction (Fig. 3., Lane D) strongly supports the notion that galactose oxidase/NaB^3H_4 method only labels the exposed surface glycoproteins. The enrichment of the radioactivity in the 52-kdalton band and 42-kdalton band in Lane D as compared to that in Lane A correlated well with the increase of specific activities of 5′-nucleotidase and alkaline phosphatase in plasma membrane fraction.

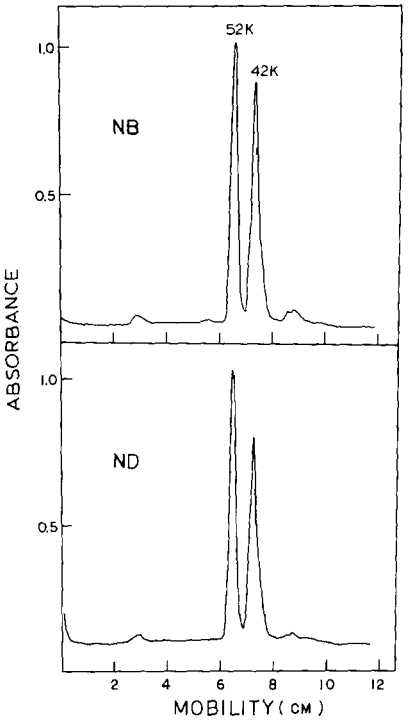

Fig. 4. Electrophoretic pattern of galactoproteins of undifferentiated (NB) and differentiated (ND) N-18 neuroblastoma cells. Both NB and ND cells were radiolabeled by the galactose oxidase/NaB^3H_4 method, plasma membrane fractions were prepared and analyzed by SDS-polyacrylamide slab gradient gel electrophoresis. Each lane contained 50 μg protein. Densitometric tracings were made from the fluorogram obtained after gel electrophoresis as described in Materials and Methods.

In addition to the 52-kdalton band and 42-kdalton band, additional labeled protein bands became visible if the gel was exposed to X-ray film for a much longer period of time. In Fig. 3, Lane D and Lane E were from the same gel except that Lane E was exposed 3-times longer than Lane D. The additional protein bands had apparent molecular weights of 220000, 45000, 40000, 31000 and 29000. It is possible that those faintly labeled protein bands were either less exposed and thus less accessible to galactose oxidase treatment or they existed in much lesser quantity.

The labeling patterns of NB and ND cells using galactose oxidase/NaB^3H_4 method were similar as shown in Fig. 4. In both cases, the 52-kdalton band and 42-kdalton band were most prominently labeled. Control experiment indicated that these two protein bands were not labeled by NaB^3H_4 without pretreatment of galactose oxidase.

Radioactive labeling of cell surface glycoprotein by the neuraminidase/galactose oxidase/NaB^3H_4 method

Surface glycoproteins with their galactosyl/*N*-acetylgalactosaminyl residues masked by sialyl groups at the non-reducing end of the carbohydrate chain are generally not accessible to galactose oxidase unless the sialyl groups are removed by neuraminidase [22]. Thus neuraminidase/galactose oxidase/NaB^3H_4 method will label surface sialylgalactosyl/sialyl-*N*-acetylgalactosaminyl glycoproteins. Fig. 5 shows the comparison of the labeling patterns of undifferentiated and differentiated N-18 cells using this method. Several points can be noted: (i) Judging from the intensity of radioactivity incorporated, eight major protein bands were labeled in both undifferentiated and differentiated cells, they had apparent molecular weights of 220000, 150000, 130000, 82000, 76000, 72000, 52000 and 42000. (ii) Among these glycoproteins, 76-, 72-, 52- and 42-kdalton bands were more prominently labeled in undifferentiated cells while 220-kdalton and 150-kdalton bands were more prominently labeled in differentiated cells. (iii) Based on the specific radioactivity in-

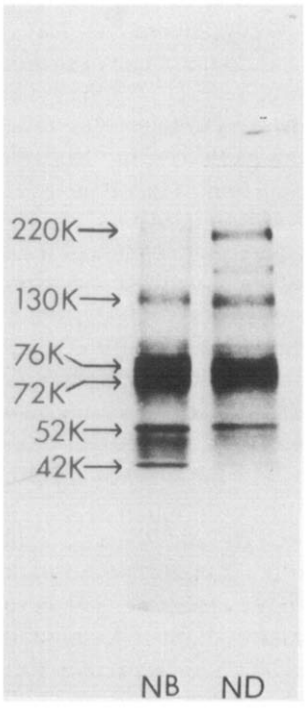

Fig. 5. Fluorograms of electrophoretic pattern of sialylgalactoproteins of undifferentiated (NB) and differentiated (ND) N-18 neuroblastoma cells. Both NB and ND cells were labeled by the neuraminidase/galactose oxidase/NaB^3H_4 method, plasma membrane fractions were isolated, and analyzed by SDS-polyacrylamide slab gradient gel electrophoresis. Each lane contained 50 μg protein. The fluorogram was made after 25 days exposure to X-ray film.

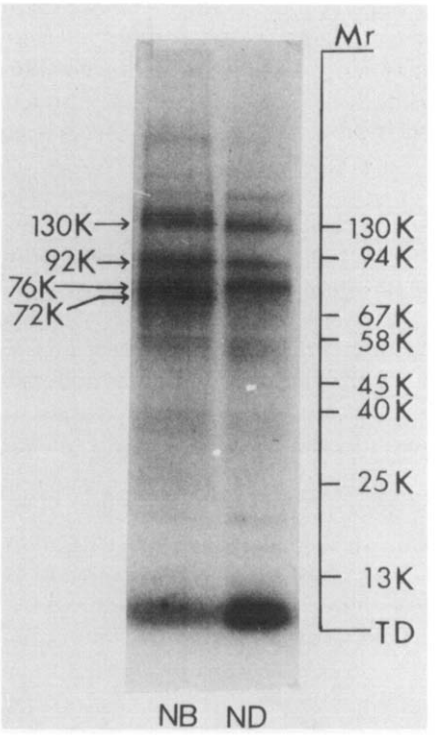

Fig. 6. Autoradiogram of electrophoretic pattern of ^{125}I-labeled proteins in undifferentiated (NB) and differentiated (ND) N-18 neuroblastoma cells. NB and ND cells were labeled by the lactoperoxidase/^{125}I method, plasma membrane fractions were isolated and analyzed by SDS-polyacrylamide slab gradient gel. Each lane contained 50 μg protein.

corporated into these protein bands as estimated from the densitometric tracings (data not shown), undifferentiated N-18 cells contained about 10–15% more sialylgalactoproteins than that of differentiated cells. A higher sialylgalactoproteins content in virus-transformed fibroblasts as compared to their normal counterparts has been reported by Gahmberg and Hakomori [22].

Lactoperoxidase catalyzed iodination

For comparative purpose, we also examined the iodination patterns of the exposed surface proteins of undifferentiated and differentiated N-18 cells.

The results shown in Fig. 6 indicated that the labeling patterns in both cells were qualitatively similar. The prominently iodinated protein bands in the undifferentiated cells had apparent molecular weights of 130000, 92000, 76000 and 72000 as compared to 150-, 130-, 92- and 76-kdalton bands in the differnetiated cells. Trudding et al. [10] reported a preferential iodination of 78-kdalton protein in the differentiated N2A neuroblastoma cells. In contrast, our results showed a 15% more iodination of 76-kdalton and 72-kdalton bands in the undifferentiated N-18 cells as compared to the differentiated cells.

Allowing 10% error range in the estimation of apparent molecular weight based on SDS-polyacrylamide gel electrophoresis [23], their 78-kdalton protein may be equivalent to our 76-kdalton plus 72-kdalton protein bands. If this is the case, the discrepancy between our observation and theirs could be due to (a) different clonal cell lines used and/or (b) different experimental conditions. We noted that they carried out iodination areaction by adding 10 μl of 88 μM H_2O_2 to the

reaction mixture at 15-s intervals over a period of 10 min. This condition may cause lipid oxidation, protein oxidation and cell lysis as has been previously reported [14,24].

Discussion

In the present study, we have developed a simple procedure for the isolation of plasma membrane fraction from N-18 neuroblastoma cells. The complete procedure could be finished within 4 h. The purity of the plasma membrane fraction was determined by increases of specific activities of membrane marker enzymes and incorporated [^{3}H]fucose.

Although surface labeling techniques and SDS-polyacrylamide gel electrophoresis have been used to characterize the plasma membrane proteins of mouse neuroblastoma cells [10–12,18], none of the reported works used fluorographic or autoradiographic method to detect the radiolabeled proteins. The conventional gel slicing and radioactivity counting method employed in those works sometimes resulted in poor resolution and high background of the labeling patterns and thus made it difficult to identify the radiolabeled proteins. Furthermore, despite the importance of surface glycoproteins in various membrane-mediated functions, only metabolic labeling method has been used to characterize the glycoproteins of undifferentiated and differentiated mouse neuroblastoma cells. We feel that since the undifferentiated and differentiated neuroblastoma cells may differ in their metabolic activities [1–3], other chemical or enzymatical labeling methods should be used to study the in situ composition of surface glycoproteins of these two cell populations.

Following this line of thinking, we employed (a) galactose oxidase/NaB^3H_4 method, (b) neuraminidase/galactose oxidase/NaB^3H_4 and (c)

TABLE II

RADIOACTIVE LABELED SURFACE GLYCOPROTEINS OF N-18 NEUROBLASTOMA CELLS

The intensity of radioactive label is estimated from the fluorographs of various electrophoretic patterns and expressed as follows: ++++, heavy label; +++, major label; ++, medium label; +, weak label; ±, very weak label; −, no label.

Band No.	M_r ($\times 10^{-3}$)	L-[^{3}H]Fucose	Galactose oxidase/ NaB^3H_4		Neuraminidase/ galactose oxidase NaB^3H_4		Lacto-peroxidase ^{125}I	
			NB	ND	NB	ND	NB	ND
1	250	+	−	−	−	−	±	−
2 [a]	220	+	+	+	±	+++	−	−
3	150	+	−	−	±	+	+	++
4 [a]	130	+	−	−	+	++	+++	+++
5 [b]	92	++++	−	−	−	−	++	+
6	82	−	−	−	+	+	−	−
7 [a]	76	−	−	−	++++	+++	++++	+++
8 [a]	72	+	−	−	++++	++	++	±
9 [c]	52	−	++++	++++	+++	++	−	−
10	50	−	−	−	+	±	−	−
11	45	±	+	+	±	±	−	−
12 [c]	42	−	+++	+++	++	±	−	−
13	40	−	+	+	−	−	−	−
14	31	−	+	+	−	−	−	−
15	29	−	+	+	−	−	−	−

[a] Bands 2, 4, 7, 8: major sialylgalactosyl/sialyl galactosaminyl glycoprotein.
[b] Band 5: major fucosyl glycoprotein.
[c] Bands 9, 12: major galactosyl/galactosaminyl glycoprotein.

lactoperoxidase/^{125}I method to radiolabel surface glycoproteins of both undifferentiated and differentiated N-18 cells. The results of our labeling studies were summarized in Table II. Due to the complexity of surface glycoproteins, evaluation was limited to those glycoproteins present in the highest quantities, as determined by surface labeling, and those readily resolved by SDS-polyacrylamide gel electrophoresis. Glycoproteins with large portions of carbohydrate might behave anomalously on SDS-polyacrylamide gel electrophoresis [25], the designation of apparent molecular weight was therefore made in an operational context. It can be concluded from the present study (Fig. 4, Fig. 5, Table II) that: (i) Band 9 ($M_r = 52000$) and Band 12 ($M_r = 42000$) were major galactoproteins in both undifferentiated and differentiated N-18 cells. (ii) Band 4 ($M_r = 130000$), Band 7 ($M_r = 76000$) and Band 8 ($M_r = 72000$) were major iodinable sialylgalactoproteins in both undifferentiated and differentiated N-18 cells. About 10–20% more radioactivity (either ^{3}H or ^{125}I) was incorporated into Band 7 and Band 8 of undifferentiated cells as compared to that of differentiated cells. (iii) Band 2 ($M_r = 220000$) was a major sialylgalactoprotein in the differentiated but not in the undifferentiated N-18 cells. It will be of interest to investigate whether Band 2 is equivalent to the glycoprotein with $M_r = 200000$ reported by Littauer et al. [11] in differentiated NIE-115 and N-18 neuroblastoma cells. (iv) The major fucosylated protein, Band 5 ($M_r = 92000$), apparently contained little or no galactose/*N*-acetylgalactosaminyl residue. Band 5 was also iodinable. (v) All the iodinated surface proteins were glycoproteins.

Recently several studies have demonstrated that a high molecular weight [^{14}C]glucosamine-labeled glycoprotein ($M_r = 200000$) of mouse neuroblastoma cells can be secreted into culture medium [26–28]. The possible co-identity of Band 2 and this substratum-attached glycoprotein is currently under investigation.

Although our reuslts did not show major differences in the labeling patterns between undifferentiated and differentiated N-18 cells, minor differences consistently occurred (Table II). Since the possible involvement of glycoproteins in cell differentiation may be a dynamic process which encompasses changes both in composition and topological distribution of surface glycoproteins, the labeling data obtained here should enable us to further study the changes of these identified glycoproteins, quantitatively and/or topologically, during the differentiation of N-18 neuroblastoma cells.

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