Biochimica et Biophysica Acta, 507 (1978) 107—118

BBA 77915

ISOLATION AND CHARACTERIZATION OF SURFACE GLYCOPROTEINS FROM L-1210, P-388 and HeLa CELLS

KUANG YU CHEN, RANDALL H. KRAMER and E.S. CANELLAKIS

Department of Pharmacology, Yale University School of Medicine, New Haven, Conn. 06510 (U.S.A.)

(Received July 1st, 1977)

Summary

A method is described that permits the rapid extraction of the cell surface glycoproteins of two murine leukemic cells, the P-388 and the L-1210 cells as well as those of the human adenocarcinoma cells, the HeLa cells.

Proof of the surface location of these glycoproteins is provided by labeling the intact cells; (a) with ^{125}I by the lactoperoxidase iodination technique; (b) with ^{3}H by the galactose oxidase-reductive tritiation method. Most of these glycoproteins were also shown to incorporate radioactive glucosamine and fucose. By these criteria as well as by the distribution of molecular weights, the surface glycoproteins of the two murine cells are indistinguishable; however, they differ markedly from the surface glycoproteins of HeLa cells. The extracts of the murine cells were shown to contain lectin receptor activity as determined by their ability to inhibit the lectin-induced agglutination of the intact cells.

Introduction

It has become increasingly appreciated that plasma membranes play a crucial role in growth regulation of mammalian cells [1]. Although a considerable amount of work has been done on the isolation and characterization of membranes from many different cell lines, very little is known about the function and properties of each individual membrane protein. On the basis of much circumstantial evidence, for example, cellular recognition [2], homing of lymphocytes [3], cellular growth regulation [4], platelet-collagen interaction [5], it has been suggested that surface glycoproteins may be involved in mediating the "social behavior" of mammalian cells. The discovery that glycophorin A, the

Abbreviations: PMSF, phenyl methyl sulfonyl fluoride; SDS, sodium dodecyl sulfate.

major glycoprotein of the erythrocyte membranes [6], is a transmembrane protein supports the notion that glycoproteins may serve as the vehicle for the transmission of environmental information from the exterior to the interior of cells [7,8].

We have shown that antiserum prepared against plasma membrane proteins can inhibit cell growth in the absence of complement. Therefore, we proposed that anti-membrane antiserum or antibody directed against a specific surface glycoprotein should constitute a unique system for the study of the regulation of cellular growth by plasma membrane [9].

We pursued this purpose by purifying plasma membranes from L-1210, P-388 and HeLa cells [9,10], followed by the isolation of glycoprotein from plasma membranes. Among various surface glycoprotein isolation methods [11—14], the lithium diiodosalicylate-phenol extraction method [14] yields the best results. The method is found to be generally useful for a number of different cell types and can be used to extract the same glycoproteins either from purified membrane preparations or, with increased yields, from the post-nuclear fraction of the cell homogenates. The glycoproteins extracted from leukemic cells were shown to inhibit the cell agglutination induced by plant lectins and by antisera produced against leukemic plasma membranes.

Materials and Methods

Radioactive ^{125}I, D-[6-^{3}H]glucosamine, L-[1,5,6-$^{3}H_3$]fucose, $NaB^{3}H_4$ were purchased from New England Nuclear, Boston, Mass. Glucose oxidase (EC 1.1.3.4) (200 units/ml), lactoperoxidase (EC 1.11.1.7) (80 units/mg), galactose oxidase (EC 1.1.3.9) (100 units/mg) and phenyl methyl sulfonyl fluoride (PMSF) were obtained from the Sigma Chemical Co., St. Louis, Mo. Fischer's medium, Joklick modified minimal essential medium, fetal calf serum and horse serum were from Grand Island Biological Co., Grand Island, N.Y. DBA/2 mice bearing L-1210 tumor cells were from Arthur D. Little, Inc., Cambridge, Mass. Lithium 3,5-diiodosalicylate was prepared from 3,5-diiodosalicylic acid (Eastman Kodak, 2166) and LiOH (Maclaster, Co.); diiodosalicylic acid was recrystallized twice from anhydrous methanol. Concanavalin A and wheat germ agglutinin were from Miles Labs., Elkhart, Ind., phytohemagglutinin A was from Difco Lab., Detroit, Mich. All other chemicals were of standard reagent grade.

Cell culture. L-1210 and P-388 cells were cultured as a suspension culture in Fischer's medium supplemented with 10% horse serum and harvested as described [9,10]. We wish to thank Dr. F.H. Ruddle, Yale University, for the HeLa-S_3 monolayer cells; these were cultured in Eagle's minimum essential medium supplemented with 10% fetal calf serum. The HeLa cells were dissociated from the culture dish with 2 mM EDTA in phosphate-buffered saline (10 mM Na_2HPO_4, 0.9% NaCl, pH 7.2) and grown as suspension cultures in spinner flasks at $2 \cdot 10^5$—$6 \cdot 10^5$/ml in Joklick minimum essential medium plus 10% fetal calf serum. Leukemic cells L-1210 and P-388 were also maintained in DBA/2 mice and harvested 6 days after the inoculation of $1 \cdot 10^6$ cells per mouse. Cells obtained from ascites fluid were washed twice with Fischer's medium and suspended in 10 volumes 0.2% NaCl at 4°C for 3 min, the suspen-

sion was then made isotonic by adding an equal volume of 1.6% NaCl; the cells were washed two more times with phosphate-buffered saline.

Iodination. L-1210, P-388 and HeLa cells were iodinated by the Phillips-Morrison lactoperoxidase-catalyzed iodination method essentially as previously described [9,10]. The final concentrations in the reaction mixture were: $1 \cdot 10^8$—$2 \cdot 10^8$/ml leukemic cells or $2 \cdot 10^7$—$4 \cdot 10^7$/ml HeLa cells, 50—100 μg/ml lactoperoxidase, 20—100 μCi/ml carrier free ^{125}I, 20 mM glucose, 50 munits/ml glucose oxidase in phosphate-buffered saline (78 mM NaCl, 57 mM Na_2HPO_4 and 18 mM KH_2PO_4; pH 7.2). The reaction was carried out at 4°C for 20 min. The reaction was terminated by dilution of the suspension with 10 volumes of cold Fischer's medium; the cells were washed three times with ice-cold phosphate-buffered saline.

Galactose oxidase-catalyzed reductive tritiation. We used Gahmberg's procedure [15] with slight modifications. Cells ($1 \cdot 10^7$ cells/ml, without neuraminidase pretreatment), were suspended in phosphate-buffered saline containing 15 units/ml of galactose oxidase, incubated at room temperature for 1 h with occasional mixing and then washed twice with phosphate-buffered saline. Tritiation was carried out at room temperature for 30 min with 0.1 mCi/ml of NaB^3H_4 (100 Ci/mol) in phosphate-buffered saline at a cell density of $3 \cdot 10^7$/ml. The cells were then washed three times with ice-cold phosphate-buffered saline.

Metabolic labeling. Both radioactive glucosamine and fucose are precursors which can be incorporated specifically into surface glycoproteins [16,17]. Cells were grown in the presence of 1—5 μCi/ml of D-[^{3}H]glucosamine or L-[^{3}H]-fucose for 48 h and harvested as described above.

Isolation of plasma membrane. Plasma membranes of L-1210 and P-388 cells were prepared as previously described [9,10]. Plasma membranes of HeLa cells were prepared essentially as described by Atkins and Summers [18].

Lithium 3,5-diiodosalicylate-phenol extraction. Purified plasma membranes were resuspended in 50 mM Tris · HCl (pH 7.5 with 2 mM PMSF present) at about 10 mg protein/ml concentration; alternatively, a cell homogenate which had been freed of nuclei by centrifugation at 2000 × *g* for 1 min was used. An equal volume of 0.6 M lithium diiodosalicylate in 50 mM Tris buffer was added to the suspension. After the mixture was stirred at room temperature for 20 min, two volumes of cold distilled water were added and mixed thoroughly at 4°C for another 20 min. The mixture was centrifuged at 105 000 × *g* for 1 h at 4°C; the clear supernatant fluid was then mixed with an equal volume of cold 50% phenol (w/v) and vigorously stirred at 4°C for 20 min. The aqueous phase after centrifugation at 4000 × *g* for 1 h in a swinging bucket rotor was exhaustively dialyzed against water and the dialyzed material was lyophilized; the product was washed three times with absolute ethanol (—20°C). Finally, the residue was suspended in water and dialyzed overnight. Any insoluble material was removed by centrifugation at 105 000 × *g* for 1 h.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Samples to be analyzed by SDS-polyacrylamide gel electrophoresis were solubilized in 50 mM Tris · HCl, pH 8.9, 2% SDS, 5% β-mercaptoethanol and 5 mM EDTA at 100°C for 3 min. The discontinuous SDS-polyacrylamide gel electrophoresis system was used as described by Laemmli [19] or the linear gradient slab gel system as

we previously described [9]. The gels were stained overnight with 0.025% Coomassie Blue in 25% 2-propanol and 10% acetic acid. Destaining was achieved by washing first in 0.0025% Coomassie Blue in 10% 2-propanol/10% acetic acid for 3 h and then by incubating in 10% acetic acid. Periodic acid-Schiff base staining method was used as described by Furthmayr and Marchesi [20]. Apparent molecular weights were determined according to the procedure of Laemmli [19]. For autoradiography, the destained gel was vacuum dried on a Whatman filter paper and exposed to X-ray film (RPX-Omat film, Eastman Kodak Co., Rochester, N.Y.). The fluorographic method of Bonner and Laskey [21] was used to detect tritium or ^{14}C-labeled components on the gel.

Results

General approach

The lithium diiodosalicylate-phenol extraction procedure is a modification of the original method introduced by Marchesi and Andrews [14]. Originally we applied this procedure to highly purified plasma membranes. Due to the low yield of plasma membrane (approx. 1 mg from 10^9 cells), the resulting product obtained from the lithium diiodosalicylate-phenol extraction was also low (approx. 50 $\mu g/10^9$ cells). In order to increase the yields, we attempted the lithium diiodosalicylate-phenol extraction on intact cells or on cell homogenates. Unfortunately, due to the release of DNA and the subsequent formation of a viscous gel, this approach was not successful. However, removal of the nuclei and extraction of the post-nuclear supernatant yielded essentially the same polypeptides as obtained when purified plasma membranes were used as starting material (see below). The yield of protein in the extract was increased approximately 5-fold. This was true for both the leukemic cells and HeLa cells.

The Coomassie Blue staining pattern of lithium diiodosalicylate-phenol extracts

Purified plasma membranes, when analyzed by SDS-polyacrylamide gel electrophoresis are usually composed of more than 50 polypeptides [9,10] (Fig. 1(a and d)). Application of the lithium diiodosalicylate-phenol extraction method to the isolated plasma membranes of leukemic cells (both L-1210 and P-388 cell lines) or of HeLa cells, yields three major peptides stainable with Coomassie Blue. They have apparent molecular weights of 60 000, 88 000 and 96 000. It might be expected that extracts from L-1210 and P-388 would be similar (Fig. 1(e and f)) since both are murine leukemic cell lines derived from DBA mice [22]. It was found, however, that the lithium diiodosalicylate-phenol extraction of HeLa cells, a human adenocarcinoma tumor cell line, yields a similar, if not identical, Coomassie Blue staining gel pattern (Fig. 1(b and c)).

Lactoperoxidase-catalyzed iodination labeling pattern

We have previously established that the lactoperoxidase-catalyzed iodination pattern of the cell surface membrane polypeptides of the L-1210 and the P-388 are very similar but differ from that obtained with HeLa cells [9].

For both the L-1210 and the P-388 cells, the major iodinated membrane polypeptides have apparent molecular weights of 220 000, 180 000, 135 000, 96 000, 88 000, 80 000, 75 000, 66 000, 60 000, 50 000, 45 000, 42 000, and

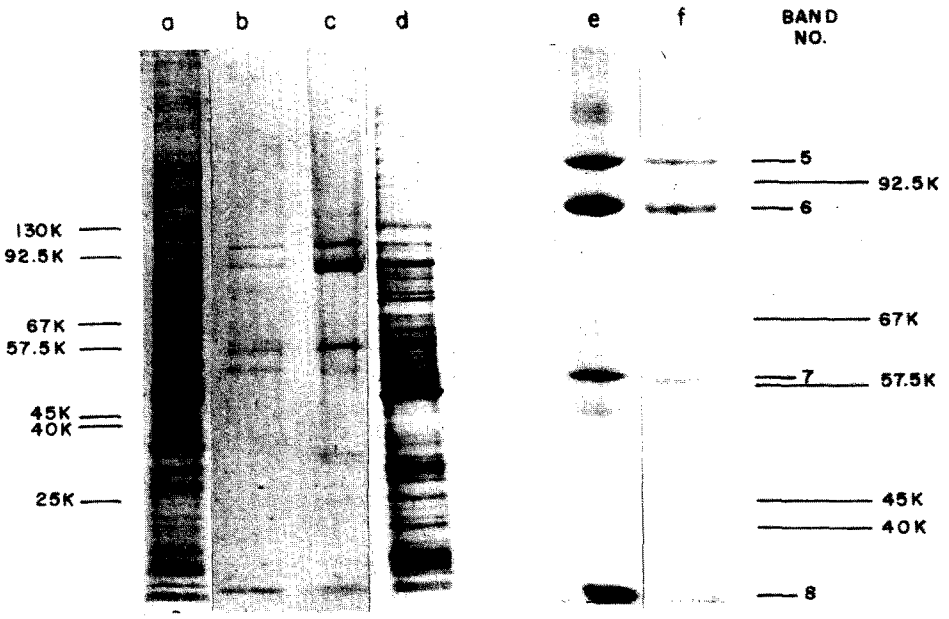

Fig. 1. Coomassie Blue-stained SDS-polyacrylamide slab gel electrophoresis pattern of membrane proteins and lithium diiodosalicylate-phenol extract. Lane a to lane d were a gradient gel (7.5—15% acrylamide, 2.7% cross-linking, 1 mm thick, 13 cm long). Lane a, membrane proteins of HeLa cells; lane b, lithium diiodosalicylate-phenol extract of post-nuclear supernatant from HeLa cells; lane c, lithium diiodosalicylate-phenol extract of post-nuclear supernatant from L-1210 cells; lane d, membrane proteins of L-1210 cells. Lane e and lane f were 15% slab gel. Lane e, lithium diiodosalicylate-phenol extract from P-388 cell membrane; lane f, lithium diiodosalicylate-phenol extract from L-1210 cell membrane.

13 000 within an estimated accuracy of ±10%. We now find that the predominant iodinated band has a molecular weight of 80 000 for which we previously reported its molecular weight at 85 000 ± 10%. Of these thirteen iodinated components, six are extractable by the lithium diiodosalicylate-phenol procedure, and have molecular weights of 220 000, 180 000, 135 000, 96 000, 88 000 and 60 000 (Fig. 2(a and b)). Of these, only the 96 000, 88 000 and 60 000 polypeptides are detectable with Coomassie Blue staining.

When the HeLa cell surface is iodinated and analyzed by SDS polyacrylamide gel electrophoresis fourteen major polypeptides are labelled. They range in molecular weight between 10 000 and approx. 250 000, with a prominent ^{125}I-labeled band at 180 000. Extraction of the iodinated plasma membranes with 0.3 M lithium diiodosalicylate solubilized between 40 and 70% of the ^{125}I label. All of the iodinated membrane proteins were equally dissociated from the membrane at this concentration of lithium diiodosalicylate as assessed by SDS-polyacrylamide gel electrophoresis. Upon extraction of the lithium diiodosalicylate-solubilized membrane with cold phenol, approx. 30% of the ^{125}I label in the lithium diiodosalicylate supernatant was found partitioned in the aqueous phase. The remainder of the radioactivity was found at the interface or in the phenol phase. Approx. 1% of the initial membrane protein was obtained in the final product derived from the aqueous phase. The SDS-polyacrylamide gel electrophoresis pattern of the ^{125}I-labeled proteins of the iso-

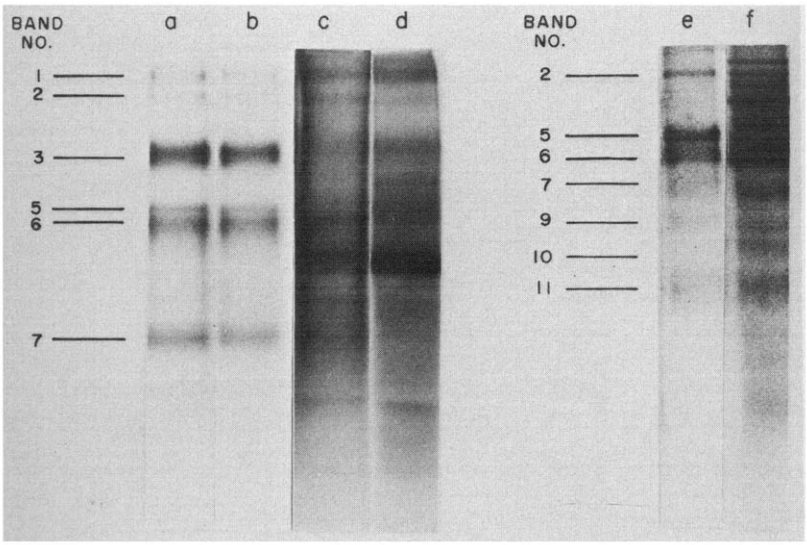

Fig. 2. Autoradiographs (^{125}I) of slab gel electrophoresis of lithium diiodosalicylate-phenol extract of leukemic cells (L-1210 and P-388) and HeLa cells. Lane a to lane d were a 7.5% gel. Lanes e and f were 6% gel. All gels were 2.7% cross-linking. Lane a, lithium diiodosalicylate-phenol extract of iodinated P-388 cell membrane; lane b, lithium diiodosalicylate-phenol extract of iodinated L-1210 cell membrane; lane c, plasma membrane proteins of iodinated P-388 cells; lane d, plasma membrane proteins of iodinated L-1210 cells; lane e, lithium diiodosalicylate-phenol extract of iodinated HeLa cell membrane; lane f, plasma membrane proteins of iodinated HeLa cells.

lated HeLa membranes and of the lithium diiodosalicylate-phenol extract is shown in Fig. 2(e and f). The major iodinated peptides in lithium diiodosalicylate-phenol extract have molecular weights of 230 000, 165 000, 140 000, 127 000, 88 000, 72 000 and 65 000. Except for the 88 000 polypeptide, the remainder of the lithium diiodosalicylate-phenol-extractable ^{125}I-labeled polypeptides were weakly, if at all, stainable with Coomassie Blue.

Carbohydrate labeling

We have applied four carbohydrate labeling methods in order to distinguish which components in the lithium diiodosalicylate-phenol extract are glycosylated. These include metabolic labeling with [^3H]glucosamine or [^3H]-fucose, the galactose oxidase-reductase tritiation and the periodate-Schiff base staining.

Glucosamine is a ubiquitous carbohydrate moiety found in plasma membrane glycoproteins [17] and was found to be a useful indicator of glycosylation in P-388, L-1210 and HeLa cells. In Fig. 3 we have presented a typical fluorogram comparing the distribution of the [^3H]glucosamine-labeled species isolated in the lithium diiodosalicylate-phenol extract of P-388, L-1210 and of HeLa cells.

In the case of lithium diiodosalicylate-phenol extract of the L-1210 cells and P-388 cells it can be seen that all of the iodinated polypeptides are also glucosamine labeled. The most heavily iodinated polypeptide, the 135 000 dalton species, also has the highest amount of the [^3H]glucosamine label. [^3H]Glucos-

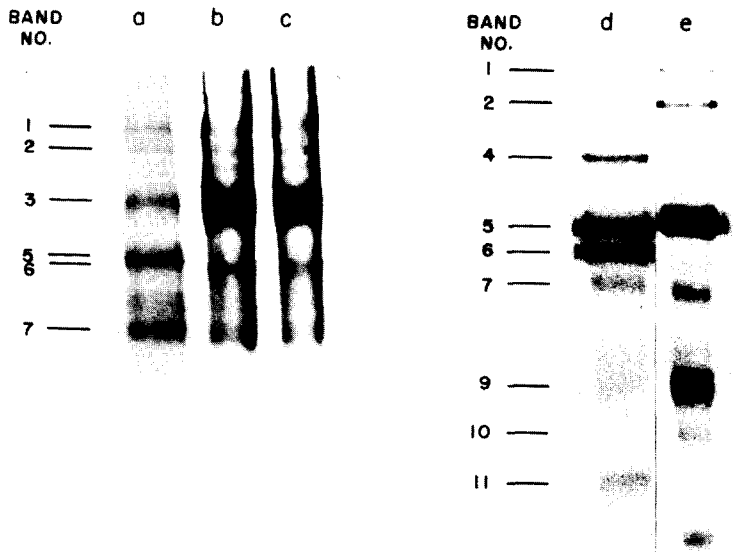

Fig. 3. Fluorograms (D-[6-^{3}H]glucosamine label) of a slab gel electrophoretic pattern of the lithium diiodosalicylate-phenol extracts of leukemic cells (L-1210 and P-388) and HeLa cells. Lane a to lane c were a gradient gel (7.5—15% acrylamide, 2.7% cross-linking). Lanes d and e were 6% glab gel, 2.7% cross-linking. Lane a, ^{125}I iodinated lithium diiodosalicylate-phenol extract of L-1210 cell membrane; lane b, [^{3}H]glucosamine-labeled lithium diiodosalicylate-phenol extract of L-1210 cell membrane; lane c, [^{3}H]-glucosamine-labeled lithium diiodosalicylate-phenol extract of P-388 cell membrane; lane d, ^{125}I-iodinated lithium diiodosalicylate-phenol extract of HeLa cell membrane; lane e, [^{3}H]glucosamine-labeled lithium diiodosalicylate-phenol extract of HeLa cell membrane.

amine labeling pattern found in the lithium diiodosalicylate-phenol extracts of L-1210 and P-388 cells, as well as the results of other labeling methods, are summarized in Table I.

In Table I, the iodinated lithium diiodosalicylate-phenol-extractable proteins of L-1210 and P-388 cells have been numbered from Band 1 to Band 8, according to their apparent molecular weight on SDS gels. These eight bands can be divided into three groups. The first group consists of Bands 1, 2 and 3 with apparent molecular weights of 220 000, 180 000 and 135 000. They are high molecular weight glycoproteins which can be labeled by fucose, glucosamine and reductive tritiation; however, they are very weakly stained by Coomassie Blue. Group 2 contains Bands 5, 6 and 7 with apparent molecular weights of 96 000, 88 000 and 60 000. They are Coomassie Blue-positive and are also glycoproteins; however, the incorporation of radioactive carbohydrate precursors into these glycoproteins is low as compared to the Group 1 glycoproteins. The third group, Bands 4 and 8, are neither iodinatable nor labeled by any of the carbohydrate labeling methods used; they are usually faintly stained by Coomassie Blue. Apparently surface immunoglobulins and histocompatibility-2 complex proteins which have molecular weights ranging from 20 000 to 70 000 are not extracted by this method. Periodic acid-Schiff base staining in purified plasma membranes or in the lithium diiodosalicylate-phenol extract from L-1210 cells were consistently weak [10].

The lithium diiodosalicylate-phenol extract obtained from the HeLa cell

TABLE I

GLYCOPROTEINS IN THE LITHIUM DIIODOSALICYLATE-PHENOL EXTRACT FROM THE PLASMA MEMBRANES OF L-121- AND P-388 CELLS

The identity of the bands labeled by various methods has been verified by using three different gel separation systems (6, 7.5 and 7.5—15% gradient). However, we cannot exclude the possibility of a multiplicity of peaks within a given band. The intensity of stain or radioactive label is expressed as follows: +++, heavy label; ++, major label; +, medium label; ±, very weak label; —, no label.

Band No.	Apparent molecular weight $\times 10^{-3}$	Coomassie Blue stain	^{125}I	[^{3}H]Glucosamine	[^{3}H]Fucose	Galactose oxidase-NaB_3H_4
1	220	—	+	+	+	+
2	180	—	+	+	+	+
3	135	—	++	+++	++	+++
4	120	±	—	±	—	—
5	96	++	+	+	+	+
6	88	+++	+	+	+	+
7	60	+	+	+	+	+
8	35	±	—	—	—	—

plasma membranes labeled with [^{3}H]glucosamine was found to contain seven major glycoproteins ranging in molecular weight from 50 000 to 350 000. One component with an apparent molecular weight of 165 000 is the predominant [^{3}H]glucosamine-labeled glycoprotein in either the purified plasma membrane or in the lithium diiodosalicylate-phenol extracts of plasma membranes. This species is exposed on the cell surface as indicated by its uptake of ^{125}I by lactoperoxidase-catalyzed iodination and by the galactose oxidase-reductive tritiation. It is also the major periodic acid-Schiff base staining band and is, in addition, labeled with [^{3}H]fucose. A summary of the components found in the lithium diiodosalicylate-phenol extract of HeLa cells is presented in Table II. It is of interest to note that a majority (seven out of nine) of the iodinated polypeptides found in the lithium diiodosalicylate-phenol extract were also labeled

TABLE II

GLYCOPROTEINS IN THE LITHIUM DIIODOSALICYLATE-PHENOL EXTRACT FROM THE PLASMA MEMBRANES OF HeLa CELLS

Band No.	Apparent molecular weight $\times 10^{-3}$	Coomassie Blue stain	^{125}I	[^{3}H]Glucosamine	[^{3}H]Fucose	Galactose oxidase-NaB_3H_4	Periodic acid-Schiff base
1	350	—	—	+	±	+	+
2	315	—	+	+	+	++	+
3	250	—	—	—	—	+	—
4	230	±	++	+	+	+	—
5	165	—	+++	++++	+	++++	++
6	140	—	±	—	+	—	—
7	127	—	+	++	—	—	—
8	96	++	±	—	—	—	—
9	88	+++	+	+	+	—	—
10	72	—	+	±	±	—	—
11	65	—	+	—	—	—	—
12	60	++	±	—	—	—	—

with [^{3}H]glucosamine. One high molecular weight band with an approximate molecular weight of 350 000 was not iodinated but was labeled with [^{3}H]glucosamine and gave a positive periodic acid-Schiff reaction. The surface localization of this component is indicated by its labeling with the galactose oxidase-reductive tritiation.

Lithium diiodosalicylate-phenol extracts inhibit cell agglutination induced by lectins

Lithium diiodosalicylate-phenol extracts from both L-1210 and P-388 cells inhibit phytohemagglutinin, concanavalin A and wheat germ agglutinin induced cell agglutination. They also inhibit the agglutination induced by heat-inactivated antiserum prepared against the purified cell membranes. For example, phytohemagglutinin A induced P-388 cell agglutination (70 μg/ml phytohemagglutinin A) can be inhibited by 12 μg/ml lithium diiodosalicylate-phenol extract from L-1210 cells.

Discussion

In developing the methods, we found that when we worked with a large number of cells (10^9 cells), removal of intact nuclei was absolutely critical in order to reduce the total volume of lithium diiodosalicylate solution that is necessary for the solutibilization of cellular membranes. On the other hand, Hunt et al. [23] recently reported the isolation of a single concanavalin A receptor from the mouse L-929 cells by first dissolving whole L-cells in 0.3 M lithium diiodosalicylate. The lithium diiodosalicykate to cell number ratio in their case was 1 mmol per 10^7 cells, i.e. approx. 300 ml 0.3 M lithium diiodosalicylate solution should be used to solubilize 10^9 cells.

Two strategies have been used to study the surface glycoproteins of mammalian cells, namely: (1) the protease digestion method, and (2) the glycoprotein solubilization method. By using the former method, only cleaved glycopeptide fractions are obtained. Walborg et al. [24,25] used papain digestion to release glycopeptides from several ascites tumor cells and showed that they inhibit agglutination by several plant lectins. Warren and his coworkers [26,27] used the trypsin and pronase digestion methods to compare the released glycopeptides of normal cells and transformed cells. However, in order to understand the disposition [28] as well as the physiological and biochemical functions [29, 30] of glycoproteins on the cell surface, it seems necessary to isolate the intact surface glycoproteins. Solubilization of cell surface glycoproteins has been achieved by using aqueous phenol [11], aqueous pyridine [12], detergent [31] and lithium diiodosalicylate [14]. Among these methods, the lithium diiodosalicylate solubilization coupled with phenol extraction has been used to selectively isolate the major glycoprotein, glycoprotein A, from human erythrocyte membranes [14]. This method, with certain modifications, has since then been applied to several other cell lines [23,32,33]. In their pioneering work with L-1210 cells, Burger and his coworkers [33] used phenol, guanidine, pyridine and lithium diiodosalicylate to solubilize the glycoproteins and reported that the lithium diiodosalicylate-extracted fractions showed four bands on SDS-polyacrylamide gel with apparent molecular weights ranging from 40 000

to 60 000. Hourani et al. [34] also used lithium diiodosalicylate to solubilize the glycoproteins from the L-1210 cell surface and reported that six proteins were extracted, among them four were glycoproteins as defined by periodic acid-Schiff base staining. These glycoproteins had apparent molecular weights of 84 000, 63 000, 44 000 and 33 000 on 6% SDS-polyacrylamide gel. The yield was approx 4—7 mg protein/5 · 10^{10} cells.

With the availability of more recent methodology we first labeled the cells with ^{125}I and demonstrated that most, if not all, of the extracted polypeptides are exposed on the cell surface. Furthermore, we have used a number of carbohydrate labeling methods, both surface labeling (galactose oxidase-reductive tritiation) and metabolic labeling, to show that indeed most of the extracted proteins are glycoproteins and they are synthesized by the cell. We have also found that the lithium diiodosalicylate-phenol extraction method will preferentially isolate cell surface glycoproteins, even from the post-nuclear cell homogenate. (Note the similarity in pattern obtained in lane c and lane f of Fig. 1 which are lithium diiodosalicylate-phenol extracts of post-nuclear supernatant and of plasma membranes from L-1210 cells, respectively.) These additional methodological refinements permit us to avoid a dependence on the Coomassie Blue stain, which does not detect all the extracted glycoproteins, or on the periodic acid-Schiff base staining, which also has limitations. For example, the substitution of an acetyl group on C_7 of sialic acid virtually inhibits oxidation of sialic acid by periodate and renders it undetectable by periodic acid-Schiff base staining method [35]. In addition, Gahmberg recently showed that, with the human erythrocytes, the periodic acid-Schiff base staining detects only three bands while the galactose oxidase plus [^{3}H]$NaBH_4$ reveals more than 20 glycoproteins [36]. In our early experiments we provided evidence that 1 mM PMSF should be present during homogenization and membrane isolation to prevent the degradative activities of endogenous proteolytic enzymes [9,10]; we have also been careful to use intact viable cells rather than frozen cells [34]. These various modifications have permitted us to increase the yields of glycoproteins to approx. 4—6% of the membrane protein [38]. In addition, the use of high pressure gas chromatography [39] has permitted us to determine that about 80% of the sialic acid in the mouse leukemic cells is in a glycolated form and that the total sialic acid content is only 0.5—0.6% by weight of the glycoprotein extracts. This low content of sialic acid in the glycoprotein extracts derived from mouse leukemic cells probably explains their relative insensitivity toward periodic acid-Schiff staining; the latter appears to be greatly dependent on the sialic acid content of glycoproteins [37]. On the other hand, it is known that the plasma membranes of HeLa cells have high sialic acid content [40].

Gahmberg and his coworkers have recently [41] applied a reductive tritiation method to study the glycoprotein patterns of different mouse lymphoid cells. They found that most of the tritiated glycoproteins of mouse lymphoid cells are of high molecular weights (>100 000). Since it has been suggested that the L-1210 cell is derived from a "B" lymphocyte precursor [42], we compared the glycoprotein pattern of the B lymphoblasts obtained by Gahmberg with the glycoprotein pattern of lithium diiodosalicylate-phenol extracts from L-1210 cells. The tritiated glycoproteins in mouse B lymphoblasts have appar-

ent molecular weights of 210 000, 180 000, 125 000, 86 000 and 77 000, which may be compared to our values from both L-1210 and P-388 cells of 220 000, 180 000, 135 000, 120 000, 96 000, 88 000 and 60 000. The correspondence of many of these bands is extremely close considering the acceptable ±10% error range in the estimation of the apparent molecular weight on the SDS-polyacrylamide gel. On the other hand, the mouse T lymphoblasts lack the 220 000 band [41].

The Coomassie Blue staining pattern in the lithium diiodosalicylate-phenol extracts from leukemic cells and HeLa cells appears very similar, Differences in the extracts from the two cell types become apparent only when other detection procedures are used, such as iodination or carbohydrate labeling methods.

Acknowledgments

We thank Ms. S.M. Tu for her excellent technical help, Mrs. B.E. Stanley for the tissue culture work and Ms. Mary Ahern for typing the manuscript. This research was supported by a U.S.P.H.S. Research Career Award to E.S.C. (5 K06 GM-03070) and American Cancer Society grant BC-75.

References

1 Pardee, A.B. (1975) Biochim. Biophys. Acta 419, 153—172
2 Kemp, R.B., Lloyd, S.W. and Cook, G. (1976) Prog. Surface Membrane Sci. 7, 271
3 Gesner, B.M. and Ginsburg, B.M. (1964) Proc. Natl. Acad. Sci. U.S. 52, 750—755
4 Burger, M.M. and Noonan, K.D. (1970) Nature 228, 512—515
5 Chesney, C., Harper, E. and Colman, R.W. (1972) J. Clin. Invest. 51, 2693—2701
6 Marchesi, V.J., Tillack, T.W., Hackson, R.H., Segrest, J.P. and Scott, R.E. (1972) Proc. Natl. Acad. Sci. U.S. 69, 1445—1449
7 Ji, T.H. and Nicolson, G.L. (1972) Proc. Natl. Acad. Sci. U.S. 71, 2212—2216
8 Yahara, I. and Edelman, G.M. (1975) Exp. Cell Res. 91, 125—142
9 Chen, K.Y., Tsai, C.M. and Canellakis, E.S. (1975) Cancer Res. 35, 2403—2412
10 Tsai, C.M., Chen, K.Y. and Canellakis, E.S. (1975) Biochim. Biophys. Acta 401, 196—212
11 Kathan, R.H., Winzler, R.J. and Johnson, C.A. (1961) J. Exp. Med. 113, 37—45
12 Blumenfeld, O.O. and Zvilichovsky, B. (1963) Arch. Biochem. Biophys. 21, 245—252
13 Hamaguchi, H. and Cleve, H. (1972) Biochem. Biophys. Res. Commun. 47, 459—464
14 Marchesi, V.T. and Andrews, E.P. (1971) Science 174, 1247—1248
15 Gahmberg, C.G. and Hakomori, S. (1973) J. Biol. Chem. 248, 4311—4317
16 Kaufman, R.L. and Ginsburg, V. (1968) Exp. Cell Res. 50, 127—132
17 Kawasaki, T. and Yamashina, I. (1971) Biochim. Biophys. Acta 225, 234—238
18 Atkins, P.H. and Summers, D.F. (1971) J. Biol. Chem. 246, 5162—5175
19 Laemmli, U.K. (1970) Nature 227, 680—685
20 Furthmayr, H. and Marchesi, V.T. (1976) Biochemistry 15, 1137—1144
21 Bonner, W.M. and Laskey, R.A. (1974) Eur. J. Biochem. 46, 83—88
22 Geran, R.I., Greenberg, N.H., MacDonald, M.M., Schumacher, A.M. and Abbott, B.J. (1972) Cancer Chemother. Rep. 3, Part 3, 7—9
23 Hunt, R.C., Bullis, C.M. and Brown, J.C. (1975) Biochemistry 14, 109—115
24 Smith, D.F., Neri, G. and Walborg, E.F., Jr. (1973) Biochemistry 12, 2111—2118
25 Wray, W.P. and Walborg, Jr., E.F. (1971) Cancer Res. 31, 2072—2079
26 Buck, C.A., Glick, M.C. and Warren, L. (1970) Biochemistry 9, 4567—4576
27 Buck, C.A., Fuhrer, J.P., Soslau, G. and Warren, L. (1974) J. Biol. Chem. 249, 1541—1550
28 Singer, S.J. (1974) Annu. Rev. Biochem. 43, 805—833
29 Nicolson, G.L. (1976) Biochim. Biophys. Acta 458, 1—72
30 Rapin, A.M.C. and Burger, M.M. (1974) Adv. Cancer Res. 20, 1—91
31 Allan, D., Auger, J. and Crumpton, M.J. (1972) Nat. New Biol. 236, 23—25
32 Merrell, R., Gotlieb, D.I. and Glaser, L. (1975) J. Biol. Chem. 250, 5655—5659
33 Jansons, V.K. and Burger, M.M. (1973) Biochim. Biophys. Acta 291, 127—135
34 Hourani, B.T., Chase, N.M. and Pincus, J.H. (1973) Biochim. Biophys. Acta 328, 530—532
35 Warren, L. (1964) Biochim. Biophys. Acta 83, 129—132

36 Gahmberg, G.C. (1976) J. Biol. Chem. 251, 510—515
37 Steck, T.L. and Fox, C.F. (1972) PAS-Sialic Acid: in Membrane Molecular Biology (Fox, C.F. and Keith, A., eds.), Sinauer Associates, Stamford, Ct.
38 Warren, L. (1959) J. Biol. Chem. 234, 1971—1975
39 Yu, R.K. and Ledeen, R.W. (1970) J. Lipid Res. 11, 506—516
40 Bosmann, H.N., Hogopian, A. and Eylar, E.H. (1958) Arch. Biochem. Biophys. 128, 51—69
41 Gahmberg, C.G., Hagry, P. and Anderson, L.C. (1976) J. Cell Biol. 68, 642—653
42 Thomas, D.B. (1972) Eur. J. Immunol. 2, 476—482