Protein and Glycoprotein Subunit Composition of Plasma Membrane from Chick Embryo Fibroblasts*

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SUMMARY

Plasma membranes, isolated from uninfected cultures of chick embryo fibroblasts, were dissociated with sodium deoxycholate and analyzed for glycoprotein and protein subunit composition by discontinuous gel electrophoresis. More than 30 protein subunits and 18 glycoprotein subunits were obtained from the A' and B' plasma membrane fractions; the membranes in the A' fraction contain almost twice as much phospholipid and cholesterol as those of the B' fraction and have a density of 1.06 versus 1.12. Electrophoretic separation profiles were similar for both types of membranes. The molecular weights of the components range from more than 220,000 to less than 22,500. Major protein subunits had molecular weights of 175,000, 95,000 75,000, 69,000, 56,000, 47,000 35,000, and 32,000. The majority of the glycoprotein subunits migrated with protein subunits of more than 70,000 molecular weight. No estimation of molecular weights was attempted for these components.IODination of whole cells yielded A' and B' membrane fractions, which were differentially labeled, with five and seven peaks of radioactivity, respectively. Some degree of differentiation between A' and B' fractions was also obtained when cells were prelabeled with radioactive glucosamine and mannoseamine. A' membrane fractions, known to contain more lipid than B' membrane, also showed a greater recovery of isotope in the lipid region of the gel than did B' membrane.

Knowledge of the composition and orientation of protein and glycoprotein subunits of the plasma membrane is critical for our understanding of the ultrastructural organization and functional properties of this cell component. There is particular interest in the composition of the plasma membranes from cancer cells, because an accepted hypothetical mechanism for carcinogenesis (1) evokes chemical or structural modifications of this membrane. On the assumption that a static alteration, i.e. not dependent on rate of synthesis or degradation, has occurred, these modifications may take many forms: (a) deletion or addition of whole molecules; (b) rearrangement or reorientation of constitutive molecules within the membrane matrix; (c) gross changes in glycoprotein or glycolipid carbohydrate moieties; or (d) monomer alterations involving deletion, addition, or linkage changes of saccharide, amino acid, or fatty acid residues. Determination of the exact nature of these membrane changes requires a more complete knowledge of the macro- and micromolecular structure and composition of both the normal and transformed cell plasma membranes than has been available heretofore with use of only the parameters of isotope incorporation or gel electrophoresis-separated proteins. By combination of several analytical approaches, a more defined view of the membrane structure and composition of normal and virus-transformed cells may be obtained.

Plasma membrane can be isolated from cultured chick embryo fibroblasts (2), fibroblasts transformed by oncogenic RNA viruses (3, 4), cultured rat liver cells (5), and chicken tumors (6) by flotation equilibrium centrifugation of particulate homogenates on continuous density gradients of sucrose. The isolated plasma membrane is obtained in two fractions, designated the A' and B', as evidenced by their buoyant density in sucrose, their lipid to protein ratios, their sialic acid and neutral sugar content, and their constitutive enzyme levels. Perdue and co-workers (3, 4, 6) have shown that, concomitant with transformation of chick embryo fibroblasts by avian sarcoma viruses, there is an increase in the neutral sugar content and a decrease in sialic acid content of the A' and B' plasma membrane fractions. Further indication of membrane structural and functional modifications resulting from transformation was the demonstrated increase in the rate of uptake of glucose. The uninfected versus virus-transformed chick embryo fibroblast is thus a good system for investigating membrane modifications occurring during the transformation process.

This paper is a report of the methodology employed and the results obtained on the analysis of the subunit composition of plasma membranes from cultured chick embryo fibroblasts. A preliminary report of a portion of this work has appeared (8). The second paper in this series (2) presents the results from studies of the subunit composition of plasma membranes from chick fibroblasts transformed by Rous sarcoma virus and by two ten-

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permeability-sensitive mutants of the Rous sarcoma virus, Ts-68 (9) and T5 (10).

**EXPERIMENTAL PROCEDURE**

**Methods**

**Cell Culture and Isolation of Plasma Membrane—** Uninfected chick embryo fibroblasts were cultured in medium containing 4% fetal calf serum for 4 days, refed on Day 4 with medium containing 5% serum, harvested on Day 5, and plasma membrane isolated as previously published procedures (5). The term homogenate refers to the particulate material obtained after the homogenization of the scraped cell suspension in either 0.15 M NaCl (saline) or Dulbecco's (11) phosphate-buffered saline and designated A'. The term mitochondria is used for 15% of the homogenate at 200,000 x g for 40 min. The two plasma membrane fractions isolated by centrifugation on continuous density gradients of sucrose were washed three times in either saline or phosphate-buffered saline and designated A' and B'.

**Isotope Labeling Conditions—** Chick embryo fibroblasts were cultured on 100-mm plastic dishes (Falcon) and labeled with 10 μCi of L-[4,5-3H]leucine per ml of leucine-deficient medium for 3 days prior to harvest. The 3H-leucine-deficient medium consisted of 10 ml of minimal essential medium less leucine (Grand Island Biological Company, Orangeburg, N. Y.) supplemented with 1 ml of regular medium containing 5.2 μg per ml of leucine. For labeling with proline, cells were cultured for 2 days prior to harvest in 10 ml of medium containing 1 μCi of [U-14C]proline.

Fibroblast cultures were labeled with [1-[14C]glucosamine, 20 μCi per plate, for 5 days prior to harvest. Fifteen percent of the cultures was used for the membrane isolation contained isotope. Mannosamine (0.42 mm final medium concentration) was added to the isotope-containing cultures to block the conversion of glucosamine to mannose by UDP-N-acetylglucosamine 1-epimerase, as suggested by Dr. A. C. Stoolmiller. Chick embryo fibroblasts were also labeled for 5 days with 25 μCi of [3-14C]acetyl-

**DISCUSSION**

Electrophoresis at 0.5 or 1.0 ma until the sample, to which bromphenol blue and sucrose had been added, was concentrated at the interface of the stacking and running gel. Electrophoresis in the running gel was at 1 or 2 ma. For samples containing more than 100 μg of protein or of large volume, the lower amperage settings proved more suitable for both stacking and separation. After electrophoresis, the gels were fixed in two changes of 10% methanol—7.5% acetic acid for a total of 14 hours. Gels were stained for protein by incubation for 2 hours at 37° in 0.05% Coomassie blue in methanol-acetic acid-water (5:1:5 v/v/v) and were de-stained by repeated changes of 7.5% acetic acid at 37-45°.

1. A. C. Stoolmiller, Department of Pediatrics and Biochemistry, University of Chicago, Chicago, Ill., personal communication.

2. The abbreviation used is: \( R_p \), relative mobility.
RESULTS

Distribution and Molecular Weight of Membrane Subunits—Discontinuous gel electrophoresis of the homogenate and of A' and B' plasma membrane fractions separates the dissociated subunit proteins and glycoproteins into characteristic and reproducible multiple bands (Fig. 1). The term subunit is defined as a reduced, sodium dodecyl sulfate-dissociated peptide or glycopeptide chain that either originated from a larger molecular weight component or existed in the membrane as a distinct monomeric species. More than 30 species of subunits with molecular weights ranging from 220,000 to 11,000 were obtained for each membrane fraction. Table I lists general molecular weight groups of subunits, the number of distinctive peptides observed within each group, the major peptide(s) present, and the assigned approximate molecular weight of each major peptide.

Plasma membrane was solubilized, subjected to electrophoresis on discontinuous disc gels, stained with Coomassie blue, and the relative mobility (RF) of the protein subunits was related to the RF of proteins of known molecular weight. The pattern of distribution of the protein subunits was arbitrarily classified into six groups. The number of bands, their approximate molecular weight range, and the average molecular weight of the major peptide(s) were established for each group. The average molecular weight for the major peptide is expressed as the mean ± the standard deviation obtained from eight experiments.

![Fig. 1. Discontinuous sodium dodecyl sulfate gel electrophoresis of homogenates and plasma membrane Fractions A' and B' isolated from cultured chick embryo fibroblasts. Fifty micrograms of a particulate homogenate (H) or of plasma membrane Fractions A' and B' from saline-(S) or phosphate-buffered saline-(P) treated cells were subjected to electrophoresis, fixed, stained with Coomassie blue, and photographed according to procedures given under "Methods." Band numbers are related to the assigned molecular weight for each identical subunit. The isolated plasma membrane from both preparations, A'S, A'-P, B'-S, and B'-P, are deficient in high molecular weight peptides, >180,000, and are concentrated with respect to the starting material in peptides of a molecular weight of 175, 99, 69, 35 and 32 × 10^3. Dependent upon the homogenizing and washing medium, certain plasma membrane peptides are increased, whereas others are decreased. In phosphate-buffered saline medium, the 69,000 and 32,000 components are increased; the 56,000 component is higher in the saline-treated membranes.

Richmond, Calif.; sodium dodecyl sulfate sequanal grade, Pierce Chemical Co., Rockford, Ill.; and Trizma base (2-amino-2-hydroxymethyl-1,3-propanediol), glycine, and basic fuchsin, Sigma Chemical Co., St. Louis, Mo.

<table>
<thead>
<tr>
<th>Group</th>
<th>Relative mobility</th>
<th>Approximate molecular weight</th>
<th>Number of peptides</th>
<th>Major peptide(s) average molecular weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0.14</td>
<td>190</td>
<td>5</td>
<td>175 ± 5.6</td>
</tr>
<tr>
<td>II</td>
<td>0.15-0.23</td>
<td>190-140</td>
<td>6</td>
<td>90 ± 5.6</td>
</tr>
<tr>
<td>III</td>
<td>0.25-0.35</td>
<td>140-75</td>
<td>7</td>
<td>75 ± 3.5</td>
</tr>
<tr>
<td>IV</td>
<td>0.36-0.51</td>
<td>75-54</td>
<td>7</td>
<td>60 ± 2.2</td>
</tr>
<tr>
<td>V</td>
<td>0.52-0.65</td>
<td>54-41</td>
<td>8</td>
<td>47 ± 0.9</td>
</tr>
<tr>
<td>VI</td>
<td>0.66-0.95</td>
<td>40-18</td>
<td>6</td>
<td>35 ± 0.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>32 ± 0.7</td>
</tr>
</tbody>
</table>

Fig. 2. The distribution of radioactive leucine and iodine-labeled plasma membrane peptides and glycopeptides. Chick embryo fibroblasts were cultured with [H]leucine or labeled externally with $^{125}$I and the plasma membrane isolated in two fractions, the A' and B'. Membrane aliquots were dissociated with sodium dodecyl sulfate, subjected to electrophoresis, stained with Coomassie blue, scanned at 550 nm (---), and sliced for the determination of radioactivity (-----) by procedures given under "Methods." a and c are leucine-labeled membrane fractions prepared in 0.15 M NaCl; b and d are $^{125}$I-labeled membrane fractions prepared in phosphate-buffered saline. a, A' membrane, data were obtained from a single gel that was subjected to electrophoresis with 112 µg of protein and 8,400 dpm; A', membrane, data were obtained from a single gel that was subjected to electrophoresis with 200 µg of protein and 18,400 dpm; c, B' membrane, data were obtained from two gels. The gel that was scanned for protein was subjected to electrophoresis with 50 µg of protein, whereas the gel that was used for isotope determination had 220 c(g of protein and 16,600 dpm; d, B' membrane, data were obtained from a single gel that was subjected to electrophoresis with 210 µg of protein and 14,100 cpm. Note the difference in the subunit composition of membrane isolated from saline-treated cells, a and c, and those from phosphate-buffered saline-treated membrane, b and d.

Distribution of Externally Labeled $^{125}$I among Homogenate and Plasma Membrane Subunits—Cultured chick embryo fibroblasts can be labeled in situ to a high specific activity with $^{125}$I by the method of Hubbard and Cohn (12). The particulate homogenate and plasma membrane fractions, the A' and B', isolated from the labeled cells had specific activities of 123,000, 97,000, and 67,500 cpm per µg of protein, respectively. The distribution of this label on gels after electrophoresis established differences
between the homogenate and the plasma membrane fractions. Bound $^{125}$I separated into both protein and lipid regions, defined, respectively, to be the gel volume above and the sample buffer volume below the bromphenol blue marker dye. In the homogenate, 80% of the $^{125}$I label was associated with protein or glycoprotein subunits (Table II). Half of that amount was with subunits of very high molecular weight. The A' and B' membrane fractions had 36 and 59%, respectively, of the label associated with the protein region of the gel. More than one-half of the label from the membrane in the A' fraction was in the lipid region of the gel (Table II), whereas only one-third of the $^{125}$I in the B' membrane fraction was found in this part of the gel.

The A' membrane fraction had five major peaks of iodinated peptides or glycopeptides (Fig. 2b). Two of these peaks have $R_f$ values that suggest they are around 180,000 to 140,000 molecular weight. Two other peaks fall within Group III subunits (Table I), and one is in Group IV. Four peaks are observed in gel regions that have conjugating proteins and glycoprotein subunits. The fifth Group IV peak migrates in a region of the gel that contains only subunits of protein and has an approximate molecular weight of 60,000. Membranes in the B' fraction have seven iodinated components (Fig. 2d); five that correspond to those observed in A' membrane; a minor band of more than 175,000 molecular weight that is unique to B'; and one of less than 56,000 molecular weight that accounted for about 4% of the incorporated label. This latter peak is also present in trace amounts in the A' membrane. Both plasma membrane fractions have a minor component of small molecular weight that migrates near the dye band.

**Fig. 3. Electrophoretic separation of iodinated and $[^{14}C]$proline-labeled subunits from untreated and collagenase-treated chick embryo fibroblasts. Cultured cells were radioactively labeled, washed with phosphate-buffered saline, and one-half of the cultures treated with collagenase and washed once more prior to solubilization in sample buffer as described under "Methods." The dissociated subunits were subjected to electrophoresis, stained, scanned, and sliced for radioactivity measurements. A, Coomassie blue gel scan of the subunits of untreated (-) and collagenase-treated (-) cells. Only the region of the gels with an $R_F$ of 0.65 to 0.3 was found to change with treatment. B, recovered $^{125}$I in the protein region of the gels from untreated (----) and collagenase-treated (------) cells. The gel of untreated sample contained 280 $\mu$g of protein and 26,000 cpm, whereas the gel of the collagenase-treated cells contained 250 $\mu$g of protein and 16,144 cpm. C, recovered $[^{14}C]$proline in the protein region of the gels from untreated (-----) and collagenase-treated (------) cells. The gel of the untreated sample contained 316 $\mu$g of protein and 8000 cpm. The gel of the collagenase-treated cells contained 280 $\mu$g of protein and 8000 cpm.**

**TABLE II**

<table>
<thead>
<tr>
<th>Gel region</th>
<th>Homogenate</th>
<th>A' fraction</th>
<th>B' fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Recovered</td>
<td>Total</td>
<td>Recovered</td>
</tr>
<tr>
<td>Stacking gel</td>
<td>3,196</td>
<td>12.7%</td>
<td>134</td>
</tr>
<tr>
<td>Protein region</td>
<td>19,907</td>
<td>79.5%</td>
<td>3,428</td>
</tr>
<tr>
<td>Dye front</td>
<td>797</td>
<td>3.1%</td>
<td>51</td>
</tr>
<tr>
<td>Lipid region</td>
<td>1,154</td>
<td>4.5%</td>
<td>5,495</td>
</tr>
</tbody>
</table>

**Nature of High Molecular Weight Component Present in Homogenate.—**The particulate homogenate from cells labeled externally in situ with iodine had a higher $^3$I specific activity than that of the plasma membrane fractions (Table II). A large percentage of this radioactivity was associated with a component that had a molecular weight >180,000 (Fig. 4B). This material is collagen-like. The subunit was labeled heavily when the fibroblasts were cultured with $[^{14}C]$proline or labeled with $^{125}$I. After collagenase treatment, a major Coomassie blue staining band with a relative mobility of about 0.05 was removed (Fig. 3A), whereas the subunit distribution of the rest of the components was unaltered. Within this same region of the gel, the radioactivity of a $^{125}$I- and $[^{14}C]$proline-labeled peptide or glycopeptide was mostly removed (Fig. 3B and C).

**Periodic Acid-Schiff-stained Subunits of Homogenate and Plasma Membrane.—**Plasma membrane subunits, present in the A' and B' fraction and stainable with periodic acid-Schiff, are found predominantly in Group II and III protein regions of the gels (Fig. 4). As shown in Table I, this corresponds to protein subunits of 75,000 to 180,000 molecular weight. Several species of glycoprotein constitute this densely stained region as evidenced by the sharp banding pattern on photographs of the gels and by the discrete peaks in the scans for dye absorbance (Fig. 4). A peak of low molecular weight material is present at an approximate $R_F$ of 0.89. Intermediate regions of the gels contain lightly stained, diffuse bands. The homogenate fraction contains subunits of more than 180,000 molecular weight stain with periodic acid-Schiff (Fig. 4). These carbohydrate-containing subunits are not present in the A' and B' plasma membrane fractions. Material migrating more rapidly than the bromphenol blue dye band stains intensely with periodic acid-Schiff. This stainable material can be eliminated from the gel by extracting aliquots of membrane with chloroform-methanol. It is very likely that this material is glycolipid.

**Labeling of Homogenate and Plasna Membrane Carbohydrate—**

![Fig. 3](https://example.com/fig3.png)

6 V. P. Wray, unpublished observations, 1972.
containing Subunits with [14C]Glucosamine and [3H]Mannosamine—Both N-acetylmannosamine and glucosamine are incorporated into carbohydrate-containing membrane components of chick embryo fibroblasts. The recovery of applied isotopes was 89%, and the efficiency of counting the respective isotopes was 48 and 61% for [3H] and [14C]. Compared with the homogenate, little radioactivity remains within the stacking gel in the plasma membrane fractions subjected to electrophoresis. In the A' fraction, 45% of the mannosamine is incorporated into molecules found with the lipid region of the gel, whereas only 31% of the glucosamine is in this component (data not presented). The isotopic labeling of lipid is higher in the A' fraction than in the B'. Conversely, B' membranes have a larger percentage of the recovered radioactivity in the protein region of the gel.

The ratio of incorporated [14C]glucosamine to [3H]mannosamine is different within the gel regions and suggests a compositional difference for these components. This is particularly apparent for the small molecular weight components of the B' membrane fraction migrating near the bromphenol blue marker dye front (data not presented).

There are distinctive differences in the patterns of isotope incorporation into the dissociated glycoproteins of the homogenate and of the plasma membrane A' and B' fractions. In the homogenate, a high molecular weight component is present at RF of 0.1 which is labeled with glucosamine to a greater extent than mannosamine (Fig. 5, a and b). Nothing comparable to it is present in the plasma membrane fractions. A dense band of periodic acid-Schiff-staining material is also found at this RF which is collagenase-sensitive. The subunits of smaller molecular weight incorporate about equal amounts of glucosamine and mannosamine with a few exceptions. The magnitude of isotope incorporation parallels the intensity of the periodic acid-Schiff staining.

The plasma membrane fractions, A' and B', are concentrated in radiolabeled glycoproteins that have mobilities of 0.15 to 0.4 (Fig. 5, c to f). These mobilities fall within Group II and III proteins (Table I) and would have molecular weights of 180,000 to 75,000. The assignment of molecular weights to these glycoproteins must be considered tentative, since it has been established that glycoproteins containing a large fraction of carbohydrate have anomalous mobilities in sodium dodecyl sulfate gel systems (21). Like the homogenate, peaks of radioactivity in the plasma membrane fractions correlate well with peaks of periodic acid Schiff staining subunits; there are no significant reproducible differences between the glycoprotein labeling patterns of plasma membrane in the A' fraction and that of the B' fraction.

The relative distribution of the two amino sugar labels also appears to be uniform with but few exceptions. In the region of RF of 0.35, mannosamine is incorporated to a somewhat greater extent than is glucosamine (Fig. 5, d and f). This difference is reproducible and may be of significance since it is a region of subunit composition that changes with virus transformation.

**DISCUSSION**

More than 30 protein and glycoprotein subunits of plasma membrane staining with Coomassie blue and periodic acid-Schiff were separated by sodium dodecyl sulfate discontinuous gel electrophoresis. The number of resolved bands depended upon gel preparation. The RF of all subunits changed with respect to several factors, particularly acrylamide stock solution and the age of the cast gels (14). These changes were controlled by the inclusion of eight standard proteins of a broad spectrum of known molecular weight with each experiment. The use of only 3 or 4 standard proteins, as other investigators have reported (12, 21-23), was found to be inadequate for accurate assessments of subunit molecular weight for the Laemmli gel electrophoresis procedure, because peptides of either high or low molecular weight had nonlinear mobility in this system (Tris-glycine-sodium dodecyl sulfate). Neville (24) has reported similar observations for borate-sulfate-sodium dodecyl sulfate-discontinuous gel electrophoresis. In the face of this problem, however, we chose to use the sodium dodecyl sulfate discontinuous gel electrophoresis system because of its reproducibility and superior resolution.

The resolved protein subunits, those having estimated molecular weights of 175, 95, 75, 69, 56, 47, 35, and 32 x 10^3, represent major components of the membrane. The majority of the glycoprotein subunits migrate with protein subunits of 180,000 to 70,000 molecular weight. The known abnormal mobility of glycoproteins in polyacrylamide gel electrophoresis precludes approximation of molecular weight estimation for these macromolecules without further experimentation with several different gel porosities (21). Unfortunately, this type of experimentation was limited by the quantities of membranes required for each gel.

Plasma membranes isolated from several types of normal and transformed cultured cells (25-31), cells taken from freshly excised organs (23, 32, 33), and very healthy cells (12, 15, 21, 34, 35) yield varying numbers of subunits of differing molecular weights. In general, for each membrane type, different major subunits are observed. One species of subunit having 46,000 to 48,000
Fig 5. The distribution of radiolabeled glucosamine and mannose among dissociated subunits of the homogenate and A' and B' fractions of plasma membrane. The plasma membrane and the homogenate from [3H]glucosamine and [3H]mannose-labeled fibroblasts were subjected to electrophoresis, sliced, and counted according to the procedures given under "Methods." A comparable treated gel was stained by the periodic acid-Schiff procedure and scanned at 560 nm. The data are presented as disintegrations per min per slice at the respective Rf of that slice (a, c, and e) and as the per cent incorporation of radioactivity of each slice. The total disintegrations per min recovered in the protein portion of the gel were taken to be 100%. These derived values (b, d, and f) take into account the variations in labeling between experiments and the differences in labeling between the protein and the lipid in each fraction. The derived values are also compared with the absorbance scan (—) of the periodic acid-Schiff-stained subunits.
molecular weight appears in plasma membranes of several rat tissues (32), BHK-21 cells (31), and mouse L cells (26). The 47,000 molecular weight subunit from the plasma membrane of cultured chick fibroblasts comigrates with dansylated, purified rabbit muscle actin (36). Yang and Perdue (37) isolated and characterized an actin-like protein from these fibroblasts, and Perdue (38) has identified it with 80-A diameter microfilaments by electron microscopy-antigen-antibody study. If this component is an actin-like protein, it could be an intrinsic part of this membrane especially as it may be used for intramembranous particle stability and sites for intercellular adhesion (38).

In the fluid mosaic model of membranes (39), it is considered probable that certain of the membrane subunits are exposed to the external milieu, some lie on the intracellular face of the cell membrane, and still others may completely traverse the membrane. Burger (40) has suggested rearrangement of cell surface molecules as a possible mechanism for cell transformation. For comparison of normal and transformed cells with respect to cell surface components, a knowledge of which membrane subunits are normally oriented toward the cell surface is essential. Labeling whole cells with radioactive iodine has proved to be a useful approach to such studies. Of the techniques available for the bonding of I 131 to membrane proteins, the completely enzymatic method of Hubbard and Cohn (12) is the one which has greatest biologic efficacy.

Plasma membrane isolated from iodinated chick fibroblasts contained five and seven peaks of radioactivity in A' and B' fractions, respectively. The major peak of radioactivity in the A' fractions had an R F of 0.5 and migrated in the region of subunits of 60,000 molecular weight. B' fraction had a greater portion of the total membrane-associated isotope incorporated into a subunit with an R F of 0.2. Because this region has comigrating protein and glycoprotein subunits, it is unclear with which type of subunit the label is associated. The glycoprotein subunits, because of their hydrophilic nature, in part lie external to the lipid bilayer in the membrane. It is very likely, then, that this component with an R F of 0.2 is a glycoprotein.

Hubbard and Cohn (27) have shown that mouse L cells also have five to six peaks of iodinated subunits. In their study, a subunit of estimated molecular weight of 85,000 accounted for 35% of the label that was attached to the isolated membranes. This major subunit was also labeled with [3H]glucosamine and [14C]glucosamine, thus it was probably of glycoprotein composition. In contrast to this study, Poduslo et al. (25), also using the mouse L cell, report as many as 12 peaks of I 131 activity. A significant portion of this activity was associated with a membrane component of approximately 230,000 molecular weight. All other peaks had approximately the same amount of label. The reasons for the differences in the results of the two research groups are as yet unclear. The differences between chick and mouse fibroblasts may of course be attributable to species variation or to culture conditions.

The quantitative measurement of glycoprotein subunits by periodic acid-Schiff staining in a manner similar to that of protein subunits with protein stains is not possible because the number of periodate-oxidizable covalent linkages, essential for the reaction to occur, is dependent on the composition and structure of the carbohydrate moiety of each glycoprotein subunit (41).

The qualitative nature of the glycoprotein subunits is also not revealed by staining. Inclusion of other parameters, such as measurement of incorporated radioactive monosaccharides to allow additional comparisons regarding the carbohydrate moieties of the electrophoretically separated glycoprotein subunits, is of utmost importance. Radiolabeled glucosamine, the most commonly utilized monosaccharide, is a key intermediate in the biosynthesis of several of the monosaccharide substituents that are present in glycoproteins and mucopolysaccharides. In studies with BHK-21 cells (a baby Hamster kidney-derived cell line), the glucosamine label in the glycoproteins was present as glucosamine, galactosamine, and sialic acid (31). Exogenous supplies of mannosamine will block conversion of glucosamine to sialic acid, thus allowing, under the proper experimental conditions, the simultaneous approximation of sialic acid and nonsialic acid saccharide composition of membrane glycoproteins. Results indicate (data not submitted) that, for arbitrarily selected molecular weight classes of glycoprotein subunits, differential labeling does occur. The significance of these findings and the importance of utilization of such differential labeling methods will be shown in a subsequent paper.

The Coomassie blue and periodic acid-Schiff staining patterns of the membranes contained in A' and B' fractions are quite similar. However, differential iodination of these two fractions, which have quite similar subunit composition, indicates there are differences in the two fractions which cannot be ascertained by subunit composition and molecular weight studies alone. Chemical analysis indicated basic compositional differences with respect to phospholipid and cholesterol, neutral sugar, and sialic acid (2, 3). Data on the distribution of glucosamine and mannosamine label indicate that there are also differences in the quantity or types of glycolipid present in these two fractions. It is as yet unclear whether A' and B' represent specialized cell surface areas or whether one is a product of the other.

Collagen-like material was found to be associated with the fibroblast cell labeled in vivo. This material labels heavily with iodine and radioactive proline, is periodic acid-Schiff-positive, and incorporates glucosamine and, to a lesser extent, mannosamine. Collagen and membrane-associated cell products are known to be produced by cells in culture (42). Use of whole cells or of plasma membrane fractions that are not completely free of adhering cell products, in labeling studies of cell plasma membrane subunits, leads to anomalous results.

In the preparation of isolated chick fibroblast plasma membrane, certain major components present at the cell surface and contained in the homogenate are removed during the isolation procedure (Figs. 1, 4, and 5). As discussed previously, one of these components is collagen-like. In addition, depending on the homogenization and washing medium (unbuffered saline versus phosphate-buffered saline containing Ca 2+ and Mg 2+), different subunits can be found associated with the isolated plasma membrane. In the presence of 0.15 M NaCl, a 56,000 molecular weight component and other components with an R F of 0.08 to 0.15 (Fig. 2, a and c) are isolated from the plasma membrane. In the presence of phosphate-buffered saline, these latter units are markedly reduced and the 32,000 molecular weight component is increased (Figs. 1, 2, b and d). It is not known whether these proteins are intrinsic or extrinsic to the plasma membrane and are lost from it or are artifically bound to it during the isolation procedure. It is necessary, therefore, to define explicitly the experimental conditions used in membrane isolation and characterization, to work with very pure...
preparations of plasma membrane, and to employ identical conditions in comparisons of plasma membranes from different species and tissues or in the study of cell membrane modifications resulting from neoplastic transformation. For this reason, attempts to relate the data reported here with those obtained by other investigators who have employed different methods of membrane isolation, solubilization, and electrophoretic conditions would be subject to error. A comparison of the results published by Smith and Crittenden (43) while this paper was in preparation is a case in point. The electrophoretic patterns of leucine-labeled protein subunits in their chick fibroblast membranes of buoyant density 1.14 obtained by Smith and Crittenden contain a major band of 42,000 molecular weight but only medium quantities of a 33,000 molecular weight subunit. The reverse is true of membranes prepared by the Perdue and Snider technique (2). The membranes of buoyant density 1.14 obtained by Smith and Crittenden contain a major band of glucosamine- and fucose-labeled high molecular weight material not observed in the B' material (buoyant density 1.13) reported in this study (Fig. 5d).

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REFERENCES

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