INCREASE OF A HIGH MOLECULAR WEIGHT PROTEIN IN PLASMA MEMBRANES OF BHK21 CELLS TRANSFORMED BY HAMSTER SARCOMA VIRUS

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SUMMARY - Electrophoretic analysis of plasma membrane fractions from BHK21/13 cells non-transformed and transformed by the B34 strain of Hamster Sarcoma Virus showed a reproducible pattern of 18 main bands among which a band corresponding to a polypeptide of nominal molecular weight 177,000 daltons, is considerably increased in membranes of transformed cells. Labelling and solubilization studies suggest that it is an integral glycoprotein.

INTRODUCTION

The changes that occur at the cell periphery following malignant transformation by oncogenic viruses have been widely documented (for a recent review, see (1). Most of these changes affect the exocytoskeleton, such as the loss or the diminution of the LETS protein (2), or the endocytoskeleton, such as the dissociation of actin cables and microtubules which occur in transformed cells (3, 4). Much less is known about the membrane proteins which are associated with the phospholipid bilayer (integral proteins) (5). Previous studies from our laboratory using freeze-fracturing (6) had shown a 2-to 3-fold increase of intramembranous particle (IMP) density after viral transformation in three cell systems including BHK21. Although several interpretations of this observation are possible, we chose to undertake a biochemical investigation of the integral hydrophobic proteins which are likely to be the main components of the particles seen in freeze fractures (7 - 9). We report here some results obtained with the BHK21 cell system, showing a significant increase of a large, presumably integral, polypeptide with a molecular weight of approximately 177,000 daltons in a transformed clone. These results were obtained by optimalizing the electrophoretic system for the resolution of polypeptides in the 100,000 to 300,000 dalton range.

MATERIALS AND METHODS

An asparagine-dependent subclone of BHK21/13 cells was used. It had the same growth properties as the untransformed population of BHK21 cells (10). Following infection of C13/8 cells with Hamster

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Sarcoma Virus (11) and cloning in agarose medium containing dextran sulphate (12), several transformed clones were isolated. One of these, HS5, was chosen for further study. It was found to be non-virus producing and highly malignant: less than 100 cells were sufficient to induce an invasive and metastasing tumor in the Syrian hamster, whereas the 50% tumorigenic dose of its untransformed counterpart was 3.10^4 cells (13).

Conditions were established to obtain a purified plasma membrane fraction from both types of cells, beginning with Warren's Zn-fixation method (14). In each experiment, approximately 2.10^8 subconfluent cells were removed, either by trypsinisation or by scraping from large plastic plate, washed twice in saline and kept for 8 min at room temperature in 1 mM $ZnCl_2$. After centrifugation, the cells were allowed to swell in distilled water and were broken in a Dounce Homogenizer in medium containing 3 mM $MgCl_2$, 10 mM Na phosphate buffer, pH 8.0. Nuclei were removed by centrifugation and the ghosts in the supernatant were concentrated on a 65% (w/w) sucrose layer. A linear sucrose density gradient (1.23 - 1.15 g/cm^3) was then formed over the ghost containing sucrose layer and centrifuged to equilibrium at 81,000 X g for 15 hours in a SW 27.1 Beckman rotor. Fractions were collected with an Isco fractionator with continuous UV absorbance recording.

The electrophoretic profile of membrane polypeptides was analyzed in polyacrylamide gels containing sodium dodecyl sulphate (SDS), using the discontinuous Tris-buffered system of Laemmli (15) with minor modifications. Separation gels were 7.5% polyacrylamide which were 0.57% cross-linked, containing 0.1% SDS.

RESULTS

The final isopycnic equilibrium centrifugation used in the purification procedure produced a profile of three peaks. Cell ghosts were concentrated in the first light peak, which was somewhat denser for HS5 cells than for C13/8 cells: HS5 cell ghosts had a buoyant density of 1.196 ± 0.005 g/cm^3 and the C13/8 ghost density was 1.186 ± 0.005 g/cm^3, obtained from 16 and 9 independent experiments, respectively.

Phase contrast and electron microscopy controls of the final membrane fraction showed it to be a homogeneous preparation of large ghosts. Some ribosomes were present in this material, but they could be washed out with 0.75M KCl, according to the conditions described for removal of non-specifically adsorbed ribosomes (16). Freeze-fracture replicas showed intramembranous particles in an aggregated state in the ghosts from both cell types (17).

Both types of membranes yielded reproducible electrophoretic profiles, containing 18 major bands (Fig.1). A prominent band of nominal molecular weight 177,000 daltons (Band 10) was consistently found in increased quantities in membranes from transformed cells. The same result was obtained in 10 independent experiments, using either trypsinised or untreated cells. In addition, a more rapidly migrating band (Band 10) from C13/8 membranes, appeared as a double peak in HS5 membranes due to

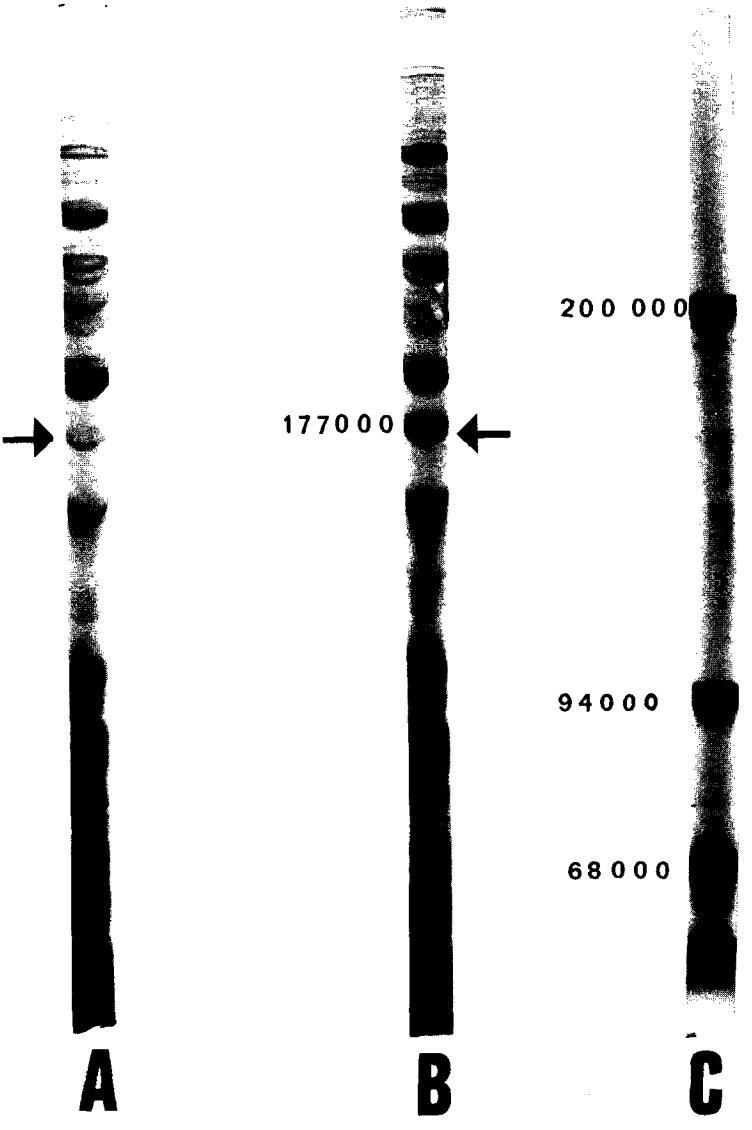
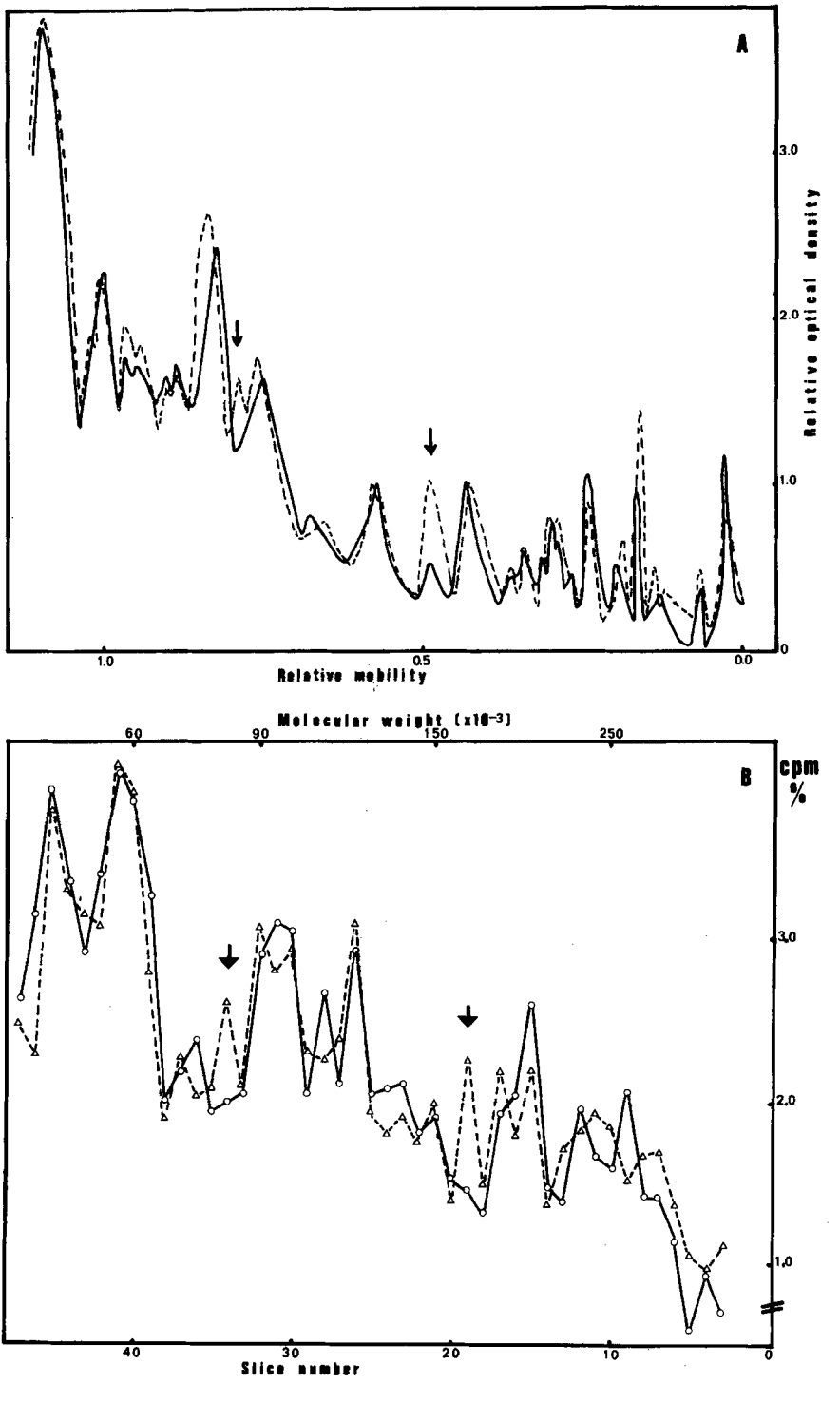


Fig. 1 Electrophoretic patterns of the membrane proteins of C13/8 and HS5 cells.

Staining was with Coomasie Blue R 250. Direction of migration in the figure is downward. Gel A: membrane fraction of C13/8 cells. Gel B: membrane fraction of HS5 cells. Gel C contained standard proteins, from top to bottom: chicken muscle myosin, rabbit muscle phosphorylase B and bovine serum albumin. Mobilities of standard proteins were reproducible within $\pm$ 4% and were linearly related to the logarithm of their molecular weights.

the appearance of a second protein having a nominal molecular weight of 94,000 daltons.

Three different criteria were used to quantitate the increase of


A
3.0
2.0
1.0
0
Relative optical density
1.0
0.5
0.0
Relative mobility
Molecular weight (x10⁻³)
60
90
150
250
B
cpm
%
3.0
2.0
1.0
40
30
20
10
0
Slice number

Band 10. Direct UV scanning (Fig.2A) led to the estimation of a three-fold increase. The optical density profile at 605 nm of eluted dye from sliced gels (18) and the radioactivity distribution of double-labelled gels led to the estimation of a two-fold increase (Fig.2B).

Band 10 could be electrophoretically distinguished from myosin and collagen components. A sample of purified HS5 membranes which was previously incubated for two hours at 37°C in presence of 175 units of collagenase Form III (Advance Biofacture Corporation) yielded the same electrophoretic profile as an untreated sample, showing that collagen-related polypeptides are not major components of the membrane fraction.

Band 10 could not be removed from the membrane preparation by a sequential EDTA (0.001 M) and KCl (0.45 M) treatment performed according to the conditions used for the extraction of actin and myosin with concomitant vesiculation from human erythrocytes ghosts (19). This treatment is also know to extract non-integral proteins from Sarcoma 180 ascitic cell ghosts (20).

Finally, HS5 cells were grown in culture medium containing ^{14}C-fucose in order to label their carbohydrate chains. Fucose-labelled membranes obtained from cells detached without trypsinisation gave a fairly simple electrophoretic radioactivity pattern with four major peaks corresponding to nominal molecular weights of 200,000, 175,000, 115,000 and 90,000 daltons (Fig.3). The second peak in stained gels corresponded to Band 10. When cells were initially detached by trypsinisation, this peak, as well as the 200,000 daltons peak, was not found in the ^{14}C-fucose electrophoretic pattern.

DISCUSSION

These experiments indicate that a significant increase of a membrane fucose-containing protein of apparent molecular weight 177,000 daltons occurs in the virus-transformed cell clone. This protein is

Fig. 2 Quantitation of the electrophoretic patterns of C13/8 and HS5 cell membrane proteins.
A: Direct UV scanning of stained gels.
Abcissa: mobility relative to bovine serum albumin, taken as 1.000.
Ordinate: optical density at 280 nm relative to that of band 9 (unvarying in both cell types) taken as an internal standard.
Solid line: C13/8; dotted line: HS5.
B: Double-labelling experiment: C13/8 and HS5 cells were grown for 48 hours in the presence of ^{14}C and ^{3}H labelled amino acids, respectively. Labelled membranes were isolated separately, then mixed and co-electrophoresed in the same gel.
Direction of migration is from right to left.
Ordinate: cpm of each gel slice as the percentage of total counts recovered.
The arrows indicate the two major differences.

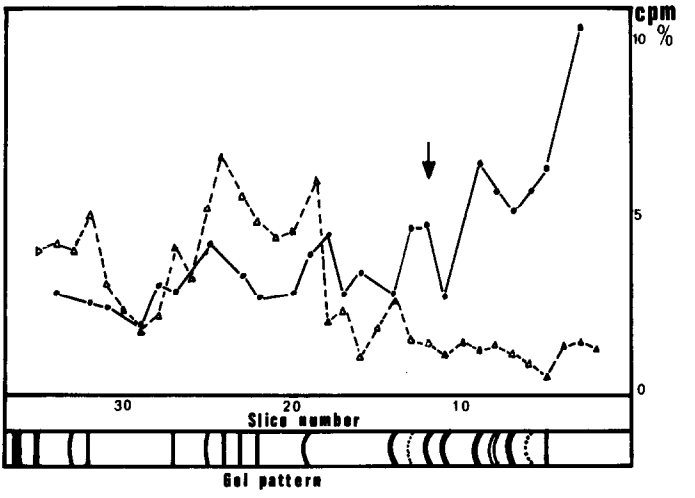


Fig. 3 Electrophoretic patterns of ^{14}C fucose-labelled membrane fractions from HS5 cells.

After electrophoresis, the gel was stained and sliced and the radioactivity in each slice was determined. The diagram below the abcissa indicates the correspondence between slices and stained bands. Continuous line (o——o) corresponds to a membrane fraction purified from scraped, non-trypsinised cells. Protection against proteolysis was achieved by adding 0.2% diisopropyl-fluorophosphate to the collection and homogenisation media. Discontinuous line (Δ---Δ) corresponds to a membrane fraction purified from trypsinised cells.

The arrow indicates the position of Band 10.

distinguishable from myosin and collagen components. The specific association of this protein with the plasma membrane is indicated by its enrichment in a purified plasma membrane preparation, compared with the total cell homogenate. Densitometry data indicate that this component represents approximately 5% of the total plasma membrane proteins, in the case of the transformed clone.

That this protein is labelled in the presence of radioactive precursors in vitro indicates that it is a product of cellular metabolism and is not a serum component which is adventitiously bound to the membrane. In addition, its labelling with fucose suggests that it is a glycoprotein. Since fucose removal upon trypsinisation does not significantly change the migration of the protein band, apparently only a small portion of the peptide sequence bound to the sugar chain seems to be exposed at the cell surface.

Finally, this protein has the characteristics of an integral protein, since it is not removed by trypsin, EDTA and KCl treatments.

Although we have no evidence that this protein is a component of the intramembranous particles seen in freeze-fractures, the parallel quantitative increase of both the protein and particles in the virus-transformed clone is striking. The increased buoyant density of plasma membranes of the transformed cells is also consistent with a higher protein/lipid ratio.

Although the electrophoretic conditions were not optimal for the resolution of low molecular weight proteins, we nevertheless detected an additional band of approximately 94,000 daltons in the profile of membrane proteins from the transformed clone. This change may be similar to those previously reported by Stone (21) and Isaka (22) in other cell systems.

As we have previously suggested (6, 17), the increased insertion of integral proteins in the plasma membrane of transformed cells may explain certain structural alterations accompanying the transformed phenotype. For instance, it could alter the anchorage of components of the exo- and the endocytoskeleton which could ultimately result in their disorganization. Moreover, if some integral proteins are involved in active transport of ions or metabolites which are critical for cell division, it is conceivable that their quantitative increase may result in altered growth properties.

We found a similar increase of the 177K protein in two other clones of BHK21 cells transformed by hamster sarcoma virus. We are currently investigating the possibility that this change is a general feature of virally and chemically transformed cells.

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