Biochimica et Biophysica Acta, 401 (1975) 386–398

BBA 77050

POLYPEPTIDE COMPOSITION OF CELL MEMBRANES FROM CHICK EMBRYO FIBROBLASTS TRANSFORMED BY ROUS SARCOMA VIRUS

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(Received December 10th, 1974)
(Revised manuscript received April 4th, 1975)

SUMMARY

Chick embryo fibroblasts were transformed by the Bryan high-titer strain of Rous sarcoma virus (RSV-BH), or a mutant (RSV-BH-Ta) inducing temperature-dependent transformation. Surface membranes from normal and transformed cells were isolated as membrane vesicles by differential centrifugation, and as cell ghosts after $ZnCl_2$ treatment and separation in an aqueous two-phase system. These preparations were analyzed by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate or phenol/urea/acetic acid. In general a greater resolution of individual bands was found in gels containing phenol/urea/acetic acid, which separates polypeptides on the bases of size and charge. Electrophoresis of preparations from nontransformed cells showed that two polypeptides (molecular weights 200 000 and 250 000) found in cell ghosts were missing in membrane vesicles.

In cell ghosts, transformation by RSV-BH resulted in a significant decrease of the 250 000 molecular weight complex. Also a polypeptide (molecular weight 73 000) prominent in membrane vesicles from nontransformed cells was decreased in transformed cells.

Surfaces from cells transformed by RSV-BH-Ta at 37 °C presented patterns similar to those for RSV-BH infected cells. Shifting these cells to 41 °C resulted in an increase in the 250 000 molecular weight complex, although the amount of this protein(s) never reached that found in noninfected cells. Inhibitors of RNA and protein synthesis failed to block the morphological changes occurring in RSV-BH-Ta cells after temperature shifts from 41 °C to 37 °C or vice-versa. The same inhibitors caused a reduction in the levels of the 250 000 molecular weight complex at both temperatures. These data indicate that these large membrane-associated polypeptides play little or no role in the morphological changes associated with transformation and its reversal.

INTRODUCTION

Transformation of cells by oncogenic viruses results in changes in their normal character which are associated with cell surface alterations. Transformed cells exhibit

loss of contact inhibition [1], increase in agglutinability by lectins [2], changes in glycolipid composition [3], alterations in transport [4], and several other modifications [5]. Alterations in the distribution of proteins and glycoproteins as a result of transformation have been observed by several authors. Bussell and Robinson [6] reported that a protein of mol. wt 142 000 was diminished in Rous sarcoma virus (RSV)-transformed cells. Wickus and Robbins [7] presented evidence that fibroblasts infected by a RSV mutant (Ts-68) inducing temperature-dependent transformation were deficient in a 45 000 mol. wt protein at the permissive temperature. The loss of a large polypeptide mol. wt 250 000 in transformed fibroblasts has been reported by Hynes [8] as well as by Hogg [9], Stone et al. [10], and Wickus et al. [11], and Vaheri and Ruoslahti [12] reported the disappearance of two proteins, mol. wts 145 000 and 210 000, after transformation of fibroblasts by RSV. In the latter case the larger protein seems to be identical to that reported by Hynes [8].

In this study we compare the proteins of plasma membranes from transformed and nontransformed chick embryo fibroblasts. Transformation of cells was induced by infection with the Bryan high-titer strain of Rous sarcoma virus (RSV-BH) and a mutant (RSV-BH-Ta) which induces temperature dependent transformation. In cells infected with this mutant morphological changes occur within 1 h after shifting from the transformation non-permissive to the transformation permissive temperature and vice-versa. The reversibility of these changes is unaffected by inhibition of DNA, RNA, or protein synthesis, suggesting that a temperature sensitive molecule participates in the morphological changes [13, 14]. The results obtained with this system are compared to those observed with non-infected and RSV-BH transformed chicken fibroblasts.

MATERIALS AND METHODS

Cells and viruses

Chick embryo cells in culture were grown in Eagle's minimal essential medium containing sodium pyruvate (5 mM) and additional glucose to give 2 g/l. This medium was supplemented with 5 % fetal bovine serum, 10 % tryptose phosphate broth (Difco), penicillin (50 unit/ml), streptomycin (50 μg/ml), tylosin (50 μg/ml), and gentamycin (10 μg/ml). Cells were infected as secondary cultures within 8 h after trypsinization and replating from primary cultures. Morphological transformation was apparent within 24–48 h after infection, and virtually all cells were transformed within 4–6 days after infection. Cultures were transferred at 2–3-day intervals, and the cells were collected by scraping from semi-confluent cultures on the second day after replating. Cultures were propagated until 6–8 weeks after infection and then discarded.

The Bryan "high-titer" strain of Rous sarcoma virus (RSV-BH), is mixed with Rous-associated virus (RAV_1, a subgroup A nontransforming avian leukosis virus) and probably a second nontransforming virus, RAVO. A mutant of RSV-BH, RSV-BH-Ta [13], also called tdBEIBH [15] transforms infected cells at 37 °C, but when placed at 41 °C, these infected cells revert to normal phenotype. RSV-BH-Ta cells also contain RAV_1 and RAVO, but the presence of these viruses has no effect on the transformation process.

Membrane preparation

Cell surface membranes were obtained by two different procedures. Plasma membrane vesicles were prepared following the procedure of Perdue and Sneider [16] for untreated cells. Homogenization was carried out in the presence of Dulbecco's phosphate buffered saline with magnesium and calcium, using a Potter-Elvehjem homogenizer with a clearance of 0.08–0.10 mm. All the studies presented in this article have been made with the B′ plasma membrane fractions [16]. Cell surfaces were also isolated by the method of Brunette and Till [17] using an aqueous two-phase polymer system. In order to avoid excessive fragmentation of the cell ghosts, homogenization was performed following the directions of Quigley et al. [18] using as medium 1 mM $ZnCl_2$. The $200 \times g$ supernatant from 2–3 homogenizations, containing cell ghosts and some cells were centrifuged at $2500 \times g$ and the pellet processed as in the original Brunette and Till method. Cell ghosts were washed 3 times with water and stored at 30 °C.

Polyacrylamide gel electrophoresis

Protein composition was analyzed by polyacrylamide gel electrophoresis in two different systems. Electrophoresis with sodium dodecyl sulfate and determinations of molecular weight were carried out as described by Dunker and Rueckert [19] in gels containing 0.1 % dodecyl sulfate and 6 M urea. Protein markers for calibration were β-lactoglobulin (18 400), ovalbumin (43 000), bovine serum albumin (68 000) (Pentex Research Products, Miles Laboratories), phosphorylase A (100 000), β-galactosidase (130 000) (Boehringer Mannheim Co.), α-chymotrypsinogen (25 700) (Sigma Chem. Corp.), spectrin subunit I (240 000) and subunit II (220 000) from human red cells. Electrophoresis in the presence of phenol/urea/acetic acid containing 2-mercaptoethanol was carried out in a system previously described [20]. The electrophoretic mobilities are expressed as relative mobilities with respect to the mobility of cytochrome *c* (M_c). Gels were fixed and stained with coomassie blue and scanned in a Gilford spectrophotometer as described elsewhere [20].

Some of the protein bands from sodium dodecyl sulfate gel electrophoresis were rerun in phenol/urea/acetic acid gels. Polypeptide bands localized by spectrophotometric scanning at 280 nm were cut, homogenized and eluted overnight with 1 % sodium dodecyl sulfate/1 % 2-mercaptoethanol at 48 °C. After a brief centrifugation, the supernatant was dialyzed against 0.05 % sodium dodecyl sulfate and lyophilized. The dry product was prepared for electrophoresis in phenol/urea/acetic acid gels as described elsewhere [20].

Analytical procedures

Protein determinations were made by the procedure of Lowry et al. [21] using bovine serum albumin as standard. Membrane samples contained 0.05 % sodium dodecyl sulfate in order to facilitate solubilization. Density of the fractions from sucrose gradients were calculated from refractive index measurements.

Chemicals and enzymes

Trypsin treated with L-(1-tosylamido-2-phenyl) ethyl chloromethyl ketone to inhibit contaminant chymotryptic activity was obtained from Worthington Biochemical Corp. Collagenase free of non collagen-specific proteolytic activity was a

gift from Dr B. Peterkofsky, Laboratory of Biochemistry, NCI, Bethesda, Maryland. All the chemicals used were at least analytical reagent grade.

RESULTS

Plasma membrane vesicles

The general electrophoretic patterns in sodium dodecyl sulfate gels for plasma membrane vesicles (Fig. 1) were similar to those presented by Wray and Perdue [22] for chick embryo fibroblasts. Comparison with the polypeptide profile of membranes isolated by Stone et al. [10] showed virtually the same proteins with the exception of some high molecular weight proteins, mol. wts 200 000 and 250 000, which were missing in the vesicles.

Membrane vesicles from RSV-BH infected cells showed a reduction of the protein region of mol. wt 70 000 to 80 000, (Fig. 1A) when compared with vesicles from noninfected cells. Membrane vesicles obtained from RSV-BH-Ta infected cells

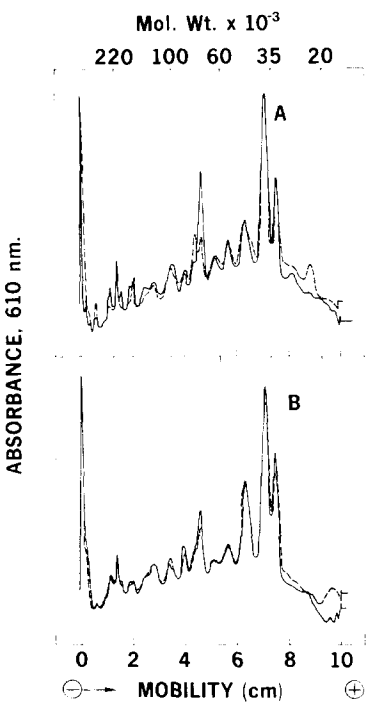

Fig. 1. Polyacrylamide gel electrophoresis of plasma membrane vesicles in sodium dodecyl sulfate and urea. 50 μg of total protein in 1 % sodium dodecyl sulfate, 1 % 2-mercaptoethanol, 6 % sucrose, was applied to 5% acrylamide gels. Gels contained 0.1 % sodium dodecyl sulfate/6 M urea. Electrophoresis was continued (6 mA/gel) until the bromophenol blue marker reached the bottom of the gel. Gels were stained with Coomassie blue under identical conditions and scanned densitometrically at 610 nm. (A) Comparison of membranes from normal chick embryo fibroblasts (——) and infected with RSV-BH (- - -). (B) Comparison of membranes from RSV-BH-Ta infected cells growing at the transformation permissive temperature, 37 °C (- - -) and at the non-permissive temperature, 41 °C (——),.

growing at the permissive (37 °C) and non-permissive (41 °C) temperature for transformation could not be distinguished (Fig. 1B). However, a 45 000 mol. wt protein, presumably actin, was present in higher proportions than those observed in vesicles from normal and RSV-BH infected fibroblasts. The 73 000 mol. wt component was present in a proportion that was intermediate between that observed in normal and RSV-BH transformed fibroblasts.

Electrophoresis of the membrane vesicles in an essentially non-aqueous system containing phenol/urea/acetic acid, and 2-mercaptoethanol revealed similar differences. Vesicles from RSV-BH transformed cells presented a decrease in a polypeptide of M_c 0.305 and an increase in a polypeptide of M_c 0.290 (Fig. 2A), compared to noninfected cells. These changes are similar to those described in sodium dodecyl sulfate systems for the proteins of mol. wts 73 000 and 78 000, respectively, suggesting

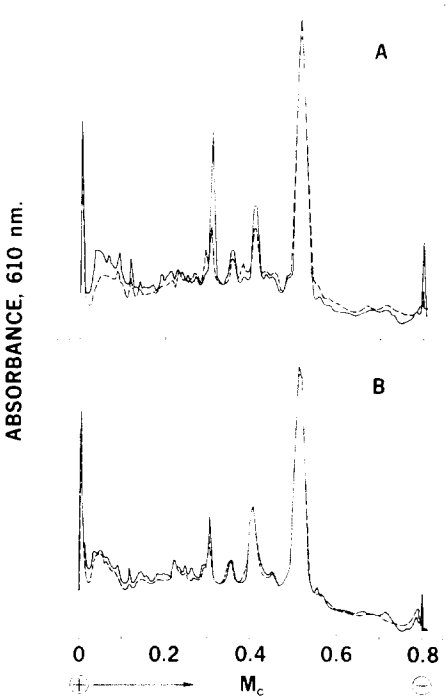

Fig. 2. Polyacrylamide gel electrophoresis of plasma membrane vesicles in a phenol/urea/acetic acid system. 40 μg of protein sample were solubilized by treatment with 0.01 M NaOH/2 % 2-mercaptoethanol for 40 min followed by addition of 4 vol. of phenol/acetic acid/urea/mercaptoethanol (45 : 25 : 30 : 2, w/v/w/v) and incubated 16–20 h at room temperature. Samples were layered over 5 % acrylamide gels equilibrated with the phenol-containing solvent. Electrophoresis, using 10 % acetic acid as tray solution, was carried out at room temperature with 2.5 mA/gel (15 V/cm) until the cytochrome *c* marker reached the bottom of the gel. Gels were stained with Coomassie blue under identical conditions and scanned densitometrically at 610 nm. The electrophoretic mobilities (M_c) are expressed as relative mobilities with respect to the mobility of cytochrome *c*. (A) Comparison of membranes from normal chick embryo fibroblasts (——) and infected with RSV-BH (- - -). (B) Comparison of membranes from RSV-BH-Ta infected cells growing at the transformation permissive temperature, 37 °C (- - -) and at the non-permissive temperature, 41 °C (— — —).

that the same protein pair is being observed in the non-aqueous system. Other minor quantitative differences were found, but it is difficult to evaluate their significance. Vesicles from RSV-BH-Ta infected cells grown at 37 °C and 41 °C, exhibited indistinguishable electrophoretic patterns (Fig. 2B). The behavior of the proteins with $M_c = 0.290$ and 0.305 parallels that observed in sodium dodecyl sulfate gel; the amount of protein with $M_c = 0.305$ (presumably the 73 000 mol. wt polypeptide) was intermediate between those observed for normal and RSV-BH transformed cells.

Cell ghosts preparations

Plasma membranes prepared by hardening of the cell surface with $ZnCl_2$ (cell ghosts) presented electrophoretic patterns different from those observed with membrane vesicles. The polypeptide profiles of the cell ghosts were similar whether obtained by densitometric scanning of stained gels, or by radioactivity measurements of gel slices after electrophoresis of polypeptides labeled in vivo with a mixture of ^{3}H-labeled amino acids (not shown). The electrophoretic patterns in the sodium dodecyl sulfate system showed the presence of two large proteins of mol. wts around 200 000 and 250 000 (Fig. 3A), which were absent from membrane vesicles. A higher proportion of 53 000 mol. wt polypeptide and a new prominent 56 000 mol. wt

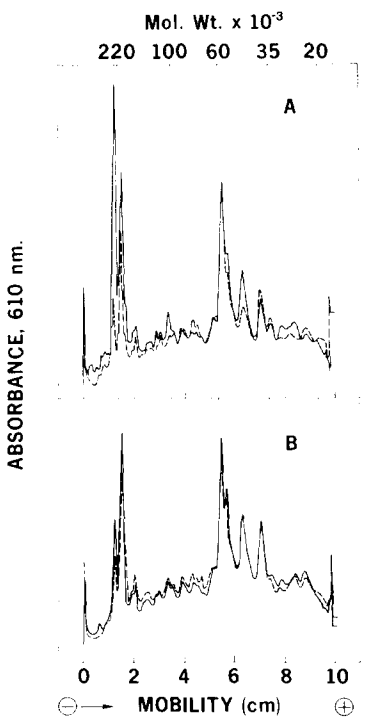

Fig. 3. Polyacrylamide gel electrophoresis of cell ghost preparations in sodium dodecyl sulfate and urea. Conditions are the same as those described in Fig. 1. (A) Comparison of cell ghosts from normal chick embryo fibroblasts (——) or infected with RSV-BH (- - -). (B) Comparison of cell ghosts from RSV-BH-Ta infected cells growing at the transformation permissive temperature, 37 °C (- - -) and at the non-permissive temperature, 41 °C (——).

polypeptide were observed in cell ghost preparations when compared with membrane vesicles. Concurrently there was a significant relative decrease in the proteins of mol. wts 73 000, 37 000 and 31 000.

Transformation of chick embryo fibroblasts by RSV-BH resulted in a loss of 250 000 mol. wt protein and some reduction of the 46 000 mol. wt component, presumably actin. The remaining proteins showed very similar distributions in normal and transformed cells (Fig. 3A).

Cell ghosts from fibroblasts infected with RSV-BH-Ta presented electrophoretic patterns (Fig. 3B) similar to those described before (Fig. 3A). The differences between cell ghosts from cells growing at the permissive (37 °C) and non-permissive (41 °C) temperatures were minimal. The 250 000 mol. wt protein was less in cells grown at 37 °C than those grown at 41 °C, but the amount of this protein produced at 41 °C by cells infected with RSV-BH-Ta was lower than that found in noninfected

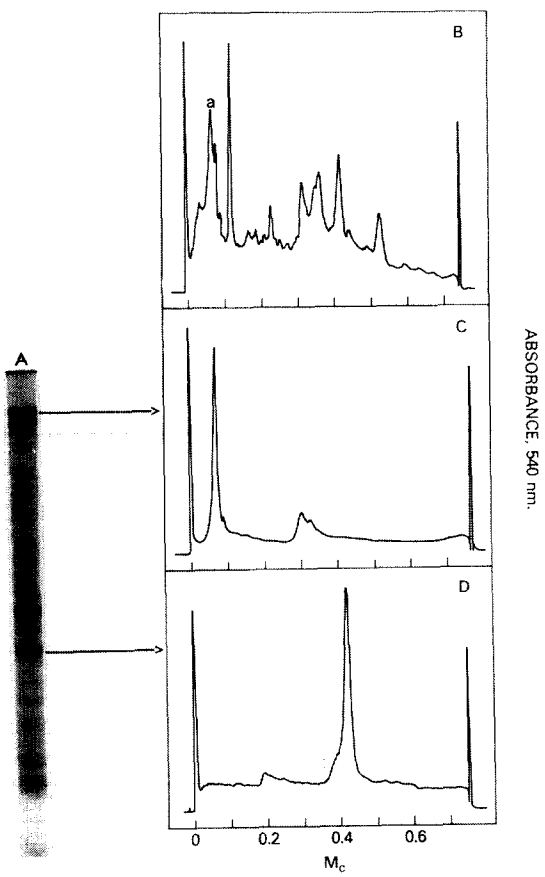

Fig. 4. Polyacrylamide gel electrophoresis in phenol/urea/acetic acid of polypeptides from cell ghosts isolated initially by sodium dodecyl sulfate gel electrophoresis. (A) Sodium dodecyl sulfate gel of cell ghosts isolated from normal cells. (B) Spectrophotometric scanning of cell ghost polypeptides resolved by phenol/urea/acetic acid electrophoresis. (C and D) Rerun in phenol/urea/acetic acid gels of 250000 and 46000 mol. wt components, respectively. Conditions are described in the text.

cells. RSV-BH-Ta infected fibroblasts showed no alteration in the distribution of 46000 mol. wt polypeptide upon temperature shift from 37 °C to 41 °C or vice-versa. In some cases the protein doublet 52 000–56 000 mol. wt was present as bands of approximately equal intensity (Fig. 3B), but usually the pattern was similar to that observed for normal or RSV-BH transformed chicken fibroblasts (Fig. 3A).

Electrophoretic analysis of cell ghosts in a system containing phenol/urea/acetic acid and 2-mercaptoethanol, revealed a more complex protein pattern. The large polypeptide (250 000 mol. wt) was resolved into a major component, "a", of low electrophoretic mobility and two minor components of higher electrophoretic mobility (Fig. 4). A strong protein doublet, M_c = 0.350 and M_c = 0.367, was present in all the cell ghosts preparations. These latter polypeptides had an inconsistent staining behavior, one protein occasionally staining weakly. A rerun of the 53 000–56 000 mol. wt proteins from sodium dodecyl sulfate gels in phenol/urea/acetic acid gels, indicates that the protein doublet (M_c = 0.350 and M_c = 0.367) is composed of polypeptide included in the 53 000–56 000 mol. wt region (not shown). The molecular weights and staining properties of these polypeptides are similar to those of δ and β subunits of microtubule proteins [23, 24]. The proteins with M_c larger than 0.4 gave a distribution somewhat similar to that observed in sodium dodecyl sulfate. Cell ghosts from RSV-BH infected cells presented a reduction of components a (mol. wt 250 000), b,

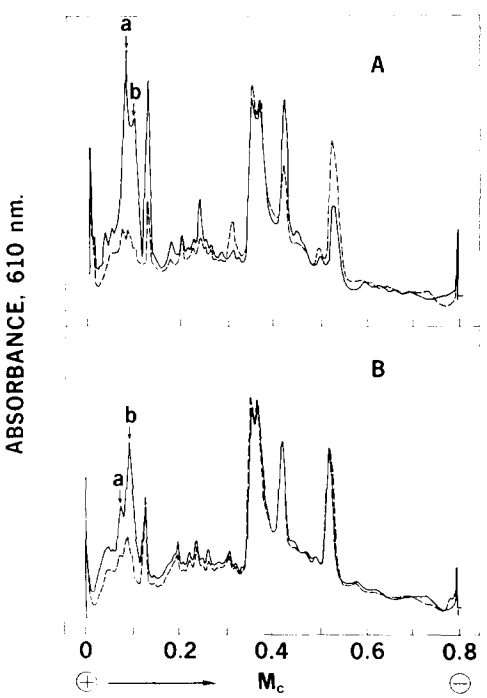

Fig. 5. Polyacrylamide gel electrophoresis of cell ghost preparations in a phenol/urea/acetic acid system. Conditions are the same as those described in Fig. 2. (A) Comparison of cell ghosts from normal chick embryo fibroblasts (——) and infected with RSV-BH (- - -). (B) Comparison of cell ghosts from RSV-BH-Ta infected cells growing at the transformation permissive temperature, 37 °C (- - -) and at the non-permissive temperature, 41 °C (——).

and polypeptides of M_c 0.125 and M_c 0.420, as compared to normal cells (Fig. 5A). The component of M_c 0.420 was identified as the 46 000 mol. wt polypeptide (Fig. 4).

Cells transformed by RSV-BH-Ta presented changes in cell ghost composition that are somewhat similar to those described above for normal and RSV-BH infected fibroblasts (Fig. 5B). The proteins with slower electrophoretic mobilities, a and b (Fig. 5B), presented the most significant changes. However, at the non-permissive (41 °C) temperature only component b showed an increase, reaching concentrations similar to those observed in normal cells. The increase in component a was considerably less marked (Fig. 5B). At the permissive temperature the changes in this region paralleled those observed in RSV-BH transformed fibroblasts.

Enzymatic treatment of chick embryo fibroblasts

Selective proteolysis combined with sodium dodecyl sulfate polyacrylamide gel electrophoresis has been used to define the protein disposition of several external

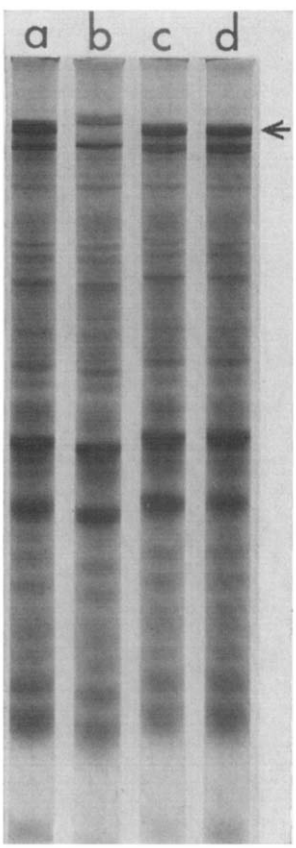

Fig. 6. Effect of enzymatic treatment before isolation of cell ghosts from normal chick embryo fibroblasts. Electrophoresis in sodium dodecyl sulfate polyacrylamide gels as in Fig. 3. (a) Phosphate-buffered saline; (b) 10 μg/ml trypsin, 10 min at 25 °C; (c) 3.3 μg/ml collagenase, 20 min at 37 °C; (d) 10 μg/ml collagenase, 90 min at 37 °C. 45 μg of protein were applied to each gel. Arrow indicates 250 000 mol. wt protein band where major changes occur.

membrane surfaces [25, 26]. Treatment of normal chick embryo fibroblasts with small amounts of trypsin, 2–10 μg/ml, resulted in a selective depletion of the 250 000 mol. wt polypeptide (Fig. 6), in agreement with previous reports [27, 28]. These results indicate that the localization of the 250 000 mol. wt polypeptide is on the external surface of the plasma membrane, a conclusion similar to that reached by other authors [8, 9] using selective proteolysis combined with lactoperoxidase-catalyzed iodination. In order to determine if this protein was collagen, cells were treated with collagenase before isolating the cell ghosts. This enzyme was shown to be free of contaminants having nonspecific proteolytic activity [29]. The cells were treated as described by Wray and Perdue [22] and under the conditions established for the total cleavage of collagen [29]. Treatment with collagenase did not alter the amount of the high mol. wt proteins (> 200 000). Previous reports [22] suggesting that the 250 000 mol. wt polypeptide was collagen or a collagen-related protein could have arisen from the use of commercial collagenase preparations, which are known to contain nonspecific proteases as contaminants [29].

Effects of antimetabolites on cell ghosts from RSV-BH-Ta transformed cells

Cells transformed with RSV-BH-Ta undergo rapid morphological changes when shifted from the permissive to non-permissive temperature or vice-versa [13]. Inhibition of cellular RNA synthesis with actinomycin D or protein synthesis with cycloheximide, does not prevent appearance of vacuoles or other morphological changes associated with transformation in RSV-BH-Ta infected cultures [14].

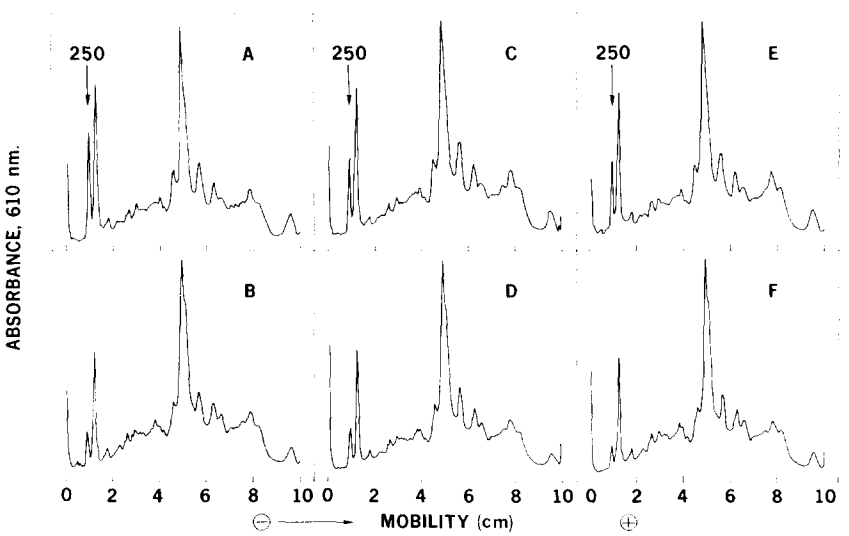

Fig. 7. Absorbance scans of cell ghost polypeptides from RSV-BH-Ta infected cells and treated with antimetabolites. Electrophoresis in 5 % acrylamide sodium dodecyl sulfate/urea gels and staining with Coomassie Blue. (A) 41 °C for 48 h; (B) 37 °C for 13 h; (C) 41 °C shifted to 37 °C for 6 h; (D) 37 °C shifted to 41 °C for 6 h; (E) 41 °C shifted to 37 °C for 6 h plus cycloheximide 2 μg/ml; (F) 37 °C shifted to 41 °C for 6 h plus actinomycin D 1 μg/ml. Culture fluids were changed before temperature shift or addition of antimetabolites.

As described earlier, cell ghosts from RSV-BH-Ta infected cells growing at 41 °C (Fig. 7A) showed a higher content of the 250 000 mol. wt protein than their counterparts maintained at 37 °C (Fig. 7B), although the cells maintained at 41 °C had a smaller amount of this protein than that present in non-infected cells. A shift in temperature from 41 °C to 37 °C for 6 h (Fig. 7C) resulted in a diminution of the 250 000 mol. wt protein. The effect of this temperature shift was the same in the presence or absence of cycloheximide (Figs 7C and 7E) but incubation of cells at 41 °C with cycloheximide for 6 h (not shown), resulted in a pattern similar to that observed for cells shifted to 37 °C. Shifting cells from 37 °C to 41 °C, with or without cycloheximide or actinomycin D (Figs 7D and 7F) allowed no apparent increase in the 250 000 mol. wt protein within 6 h. The distribution of the other proteins in the cell ghosts did not differ at either temperature in the presence or absence of antimetabolites (Fig. 7). These data indicate that in cells infected with RSV-BH-Ta, the morphological and some physiological changes are not correlated with the presence or absence of the 250 000 molecular weight component.

Electrophoretic studies of the protein composition of whole chick embryo fibroblasts, normal and transformed, gave results generally similar to those observed with cell ghosts. The 250 000 mol. wt protein was reduced after transformation by RSV-BH, and cells infected with RSV-BH-Ta and maintained at 37 °C showed smaller amounts of this large polypeptide than when maintained at 41 °C. Levels of this protein were reduced in all cells treated with cycloheximide or actinomycin D.

DISCUSSION

Cell surfaces from chick embryo fibroblasts, non-infected or infected with RSV-BH or its mutant RSV-BH-Ta [13], were isolated by two different methods and their protein compositions analyzed. Membrane vesicles were separated by their buoyant density in sucrose gradients, and cell ghosts were isolated after hardening of the cell surface with $ZnCl_2$.

Comparison of the protein patterns of membrane vesicles with those of cell membrane preparations of Stone et al. [10] revealed similar polypeptide distributions with the exception of the 200 000–250 000 mol. wt proteins. These two large polypeptides were missing from the membrane vesicles. Cell ghost preparations in comparison to membrane vesicles presented additional proteins and quantitative differences. Several of these proteins probably derive from material that underlies the membrane [30] and from loosely attached surface proteins fixed by the $ZnCl_2$ treatment. Cell ghosts presented two large molecular weight proteins, 250 000 and 200 000, a large peak formed by two proteins of 56 000 and 53 000 mol. wt, and a 45 000 mol. wt protein. Selective proteolysis has shown that the 250 000 mol. wt polypeptide is mainly a surface component, identified as the surface protein labeled by lactoperoxidase-catalyzed iodination which is also removed by proteolysis [8, 9]. The nature of the 200 000 mol. wt polypeptide is unknown, although recent reports of myosin associated with cell membranes [31, 32], raises the possibility that this protein is myosin. It is difficult to decide if the 200 000 polypeptide must be considered a membrane protein or a contaminant of cytoplasmic origin. Wallach and Winzler [26] have proposed that besides the "core" proteins, which can be removed from membranes only by drastic treatments, there are a number of proteins presenting

different degrees of membrane affinity. One could postulate that the 250 000 and 200 000 mol. wt polypeptides are not core proteins, but that their membrane affinities are significantly higher than that of other cytoplasmic proteins.

The doublet formed by polypeptides of 56 000 and 53 000 mol. wt showed peculiar staining properties. The molecular weights and the behavior of these proteins toward dyes are typical of tubulins [23, 24]. However, the occurrence of membrane-associated tubulin is questionable [33] and its possible presence in membranes isolated with $ZnCl_2$ needs confirmation. The 45 000 mol. wt protein has been observed in similar preparations [7, 34] and also is present in isolated membrane vesicles. Recent work [34] suggests that this protein is actin, although other types of proteins are not excluded.

Comparison of membrane vesicles from normal and RSV-BH transformed cells showed only one significant change; upon transformation a 73 000 mol. wt protein was significantly reduced. This change differs from the results of Stone et al. [10] who reported an increase of the same protein in transformed cells. Moreover we have been unable to find the other changes described by the same group [10]. These differences may be due to the different procedure used in the preparation of vesicles. Membrane vesicles from fibroblasts infected with RSV-BH-Ta and maintained at 37 °C or 41 °C showed no significant differences, and the amount of 73 000 mol. wt protein was intermediate between those observed for normal and RSV-BH transformed fibroblasts. Somewhat similar changes in the protein patterns were observed in the phenol/urea/acetic acid system.

Cell ghost preparations presented the most noticeable differences between the normal and transformed state. RSV-BH transformed fibroblasts lose protein(s) of molecular weight approx. 250 000. This change agrees with previous reports indicating loss or reduction of this protein upon transformation [8–10]. Fibroblasts infected with RSV-BH-Ta and maintained at 37 °C presented a reduction of this protein similar to that observed for RSV-BH transformed fibroblasts. At 41 °C larger amounts of the 250 000 mol. wt protein were seen, but the levels observed in normal cells were rarely attained. Analysis of the same preparations in phenol/urea/acetic acid revealed a selective effect on the changes of components a and b in cell ghosts. Cells infected with RSV-BH-Ta and shifted from 37 °C to 41 °C, the non-permissive temperature for transformation, regained mainly component b after extended incubation (24 h or longer) but component a (250 000 mol. wt) remained low.

Morphological changes characteristic of transformation, and the reversal of these changes to the non-transformed phenotype, can occur in the presence of actinomycin or cycloheximide, as can changes in cellular water content [35]. Synthesis and accumulation of the large membrane-associated proteins is prevented by Actinomycin D or cycloheximide in RSV-BH-Ta cells shifted from 37 °C to 41 °C. Also, RSV-BH-Ta cells maintained at 41 °C and treated with cycloheximide lose the large polypeptides without inducing morphological transformation. Clearly, the large membrane-associated proteins play little or no role in the morphological changes associated with transformation or its reversal. Where the alterations in membrane-associated proteins fit in the sequence of events related to malignant transformation remains to be determined.

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