IDENTIFICATION OF THE MAMMARY TUMOR VIRUS ENVELOPE GLYCOPROTEIN (gp52) ON MOUSE MAMMARY EPITHELIAL CELL SURFACE*

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Received April 21,1977

SUMMARY

Lactoperoxidase radioiodination of mammary epithelial cells cultured in monolayers followed by SDS-PAGE analysis revealed only a few distinct peaks. One of these, identified as major envelope glycoptrotein (gp 52) of MTV, is present on the surface of mammary epithelial cells (both tumor and normal) from chronically infected BALB/cfC3H mice but not on the surface of normal mammary epithelial cells from virus-free BALB/c mice. Its presence on the cell surface is influenced by both hormones and cell density, the same factors which greatly control the production and release of intact MTV virions into culture media. This suggests a correlation between abundance of radioiodinatable gp 52 on the cell surface and MTV found in culture media.

INTRODUCTION

Expression of the C-type viral envelope glycoprotein (gp 71) on the cell surface from various tissues has been extensively studied (1), but there is only limited information on the cell surface localization of the B-type viral envelope glycoprotein (gp 52). MMTV antigens on the surface of mouse mammary epithelial cells have been detected by immunoelectron microscopy (2,12,13,17) and immunofluorescence (10,11) as well as in various lymphoid tissues (4,5,9, 10,11). We have investigated the MTV envelope glycoprotein (gp 52) on the cell surface of tumor (BALB/cfC3H) and normal (BALB/cfC3H and BALB/c) mammary epithelial cells in monolayer culture using the lactoperoxidase radioiodination method. This method was chosen since it is known that gp 52 in intact MTV can be labeled (3,18) and no reported attempt has been made to apply this technique to label gp 52 on the cell surface of normal and tumor mammary epithelial cells.

*Abbreviations used: MMTV, mouse mammary tumor virus; SDS-PAGE, sodium docecyl sulfate polyacrylamide gel electrophoresis; DME, Dulbeccos's modified Eagle's.

ISSN 0006-291X

MATERIALS AND METHODS

Cell culture. Primary tumor cultures were obtained from spontaneous mammary tumors that developed in MTV-infected multiparous BALB/cfC3H female mice. Primary normal cultures were obtained from mammary glands from 10 to 13 day mid-pregnant mice of MTV-infected BALB/cfC3H and virus-free BALB/c strains. Mammary tumors were dissociated according to the trypsinization procedure previously described (16). Normal mammary glands were dissociated according to the collagenase digestion procedure recently described (8). The dissociated cells were seeded in DME medium with 10% FCS, 100 U of penicillin per ml, and 100 µg of streptomycin per ml at a density of 10^6 per cm^2 in 35 mm tissue culture dishes. After two days, serum-free DME medium with 10 µg/ml insulin and 5 µg/ml hydrocortisone was added.

Lactoperoxidase radioiodination. The monolayer cultures were radioiodinated two to four days later according to the procedure previously described (14) with the following modification. After several washes of the monolayer with Hanks' BSS, the reaction was started by addition of 0.012% H_2O_2 (10 µl every 5 minutes for several times) to 0.5 ml of Hanks' BSS containing 100-200 µCi carrier free $Na^{125}I$ (New England Nuclear, NEZ-033) and 50 µg of lactoperoxidase (Cal Biochem). The cells were than washed, scraped off the dishes, and then homogenized in 1 ml of 1% Triton-X solution (10 mM Tris-HCl, pH 7, 14 mM NaCl). The supernatant obtained after centrifugation at 10,000 g for 10 minutes were used for either immunoprecipitation or TCA precipitation. Preparation and absorption of anti-MTV serum and subsequent immunoprecipitation was carried out according to the procedure previously described (7). Immunoprecipitate and TCA precipitate (after several washes with ether) were solubilized with urea, SDS, and mercaptoethanol at a final concentration of 5 M, 0.1% and 2% respectively, and the mixture was heated to 100° C for a few minutes. The electrophoresis was carried out on 10 or 12.5% polyacrylamide gels at 2.5 V/cm for 18-22 hours as previously described (7).

Reverse transcriptase assay. MTV production was monitored by duplicate determinations (which differed by not more than 10%) of reverse transcriptase activity in approximately 6 ml of tissue culture fluid pooled from a 24-hour incubation of triplicate 35-mm dishes. The supernatant reverse transcriptase activity is a specific indicator of MTV production as reported previously (6) by showing an association of the viral activity with physical, morphological and immunological properties of MTV. Details of the assay have been recently described (6,19). Assays were performed under conditions where the incorporation of ^3H-dGTP was directly proportional to the concentration of MTV in the reaction mixture and to incubation time. The presence of type C virus was monitored by replacing 10 mM $MgCl_2$ with 0.5 mM $MnCl_2$, and no evidence of significant type C virus production was noted.

RESULTS AND DISCUSSION

Lactoperoxidase radioiodination of mammary epithelial cells cultured in monolayers followed by solubilization and SDS-PAGE analysis revealed only a few distinct peaks, as shown in figure 1. This suggests that there is only a limited amount of exposed tyrosine residues of membrane proteins. One of these migrated to the same position as gp 52, the major envelope glycoprotein of MTV. This radioiodinatable protein was present on the surface of mammary epithelial cells (both tumor and normal) from chronically infected BALB/cfC3H

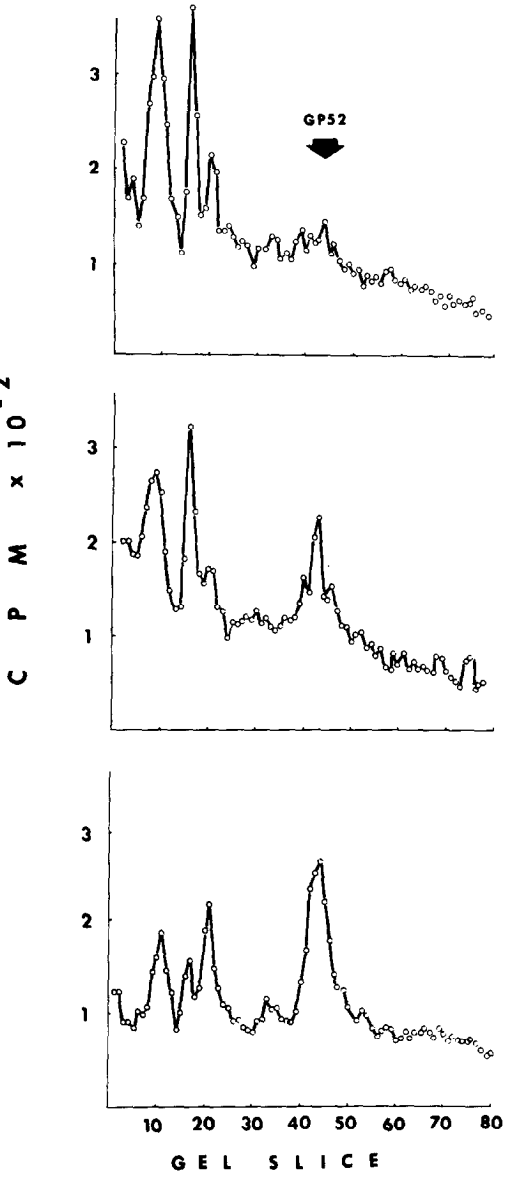

Figure 1
Electrophoretic profiles of radioiodinated cell surface proteins of cultured mammary cells: (A) BALB/c normal mammary gland (top), (B) BALB/cfC3H normal mammary gland (middle), and (C) BALB/cfC3H mammary tumor (bottom). Monolayer cultures were radioiodinated solubilized, and TCA precipitated as described in Materials and Methods. The arrow indicates the position of gp 52 determined from MTV proteins electrophoresed in a parallel gel. MTV production was monitored by duplicate determination of reverse transcriptase activity in approximately 6 ml of tissue culture fluid from a 24 hour incubation of triplicate 35 mm dishes pooled just prior to iodination: (A) 166 cpm, (B) 7233 cpm, and (C) 16,746 cpm. Background count of about 50 cpm has not been subtracted.

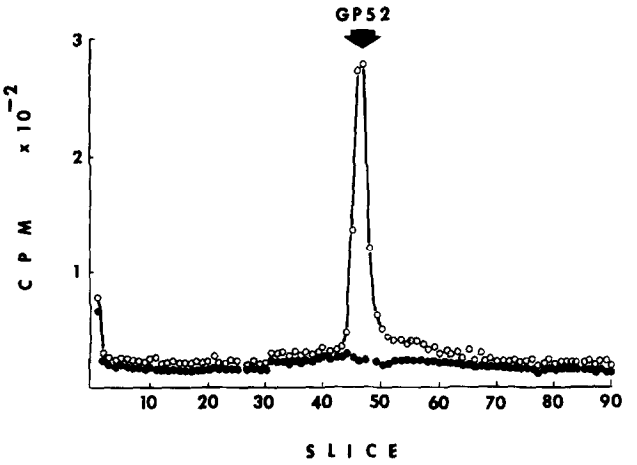

Figure 2
Electrophoretic profile of radioiodinated BALB/cfC3H mammary tumor cells after being solubilized and immunoprecipitated with either anti-MTV serum (○) or normal rabbit serum (●). The arrow indicates the position of gp 52.

mice but not on the surface of normal mammary epithelial cells from virus-free BALB/c strain of mice. This is consistent with the observation that cultured cells obtained from the mammary gland, whether normal or tumor, of BALB/cfC3H mice produced MTV, whereas cultured cells from BALB/c mammary gland did not produce any detectable amount into the culture medium.

Further studies on the nature of this radioiodinatable protein migrating to the same position as gp 52 and factors affecting its presence on cell surface were carried out using the monolayer cultures of BALB/cfC3H mouse mammary tumor cells. Labeled monolayer was solubilized and treated with anti-MTV serum The immune-precipitate was subsequently analyzed on SDS-PAGE and the profile showed a single distinct peak migrating to the same position as gp 52, as shown in figure 2. The specificity of the immunoprecipitation was shown by the treatment of the same extract with normal rabbit serum and SDS-PAGE analysis which revealed no peak. This indicates that the radioiodinatable cell surface protein migrating to the same position as gp 52 has the antigenic property of gp 52 of MTV.

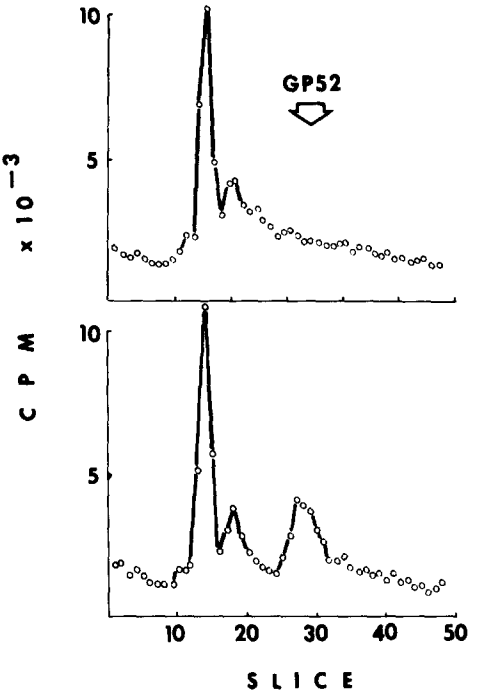

Figure 3
Effect of hormones on the electrophoretic profile of radioiodinated cell surface proteins of BALB/cfC3H mouse mammary tumor cells. (A) 10 µg/ml insulin alone (top), and (B) 10 µg/ml insulin and 5 µg/ml hydrocortisone (bottom). Monolayer cultures were radioiodinated, solubilized, and TCA precipitated as described in Materials and Methods. The arrow indicates the position of gp 52. High molecular weight proteins are resolved into only two peaks (as compared to three peaks in Figure 1) because the time electrophoresed was about half as long as in Figure 1. Reverse transcriptase activity in culture medium pooled prior to iodination is as follows: (A) 1674 cpm, (B) 13,571 cpm. Background count of about 50 cpm has not been subtracted.

Since it is known that hormones and cell density greatly influence production of MTV into the culture medium (6,15,16), we examined to see whether these factors also influence radioiodinatable gp 52 on the cell surface. Figure 3 shows profiles of radioiodinated surface proteins from mammary epithelial cells cultured in the presence of insulin alone and from those cultured in the presence of both insulin and hydrocortisone. Cells cultured in the presence of hydrocortisone consistently revealed radioiodinatable gp 52 whereas those cultured in the absence of hormone showed either undetectable

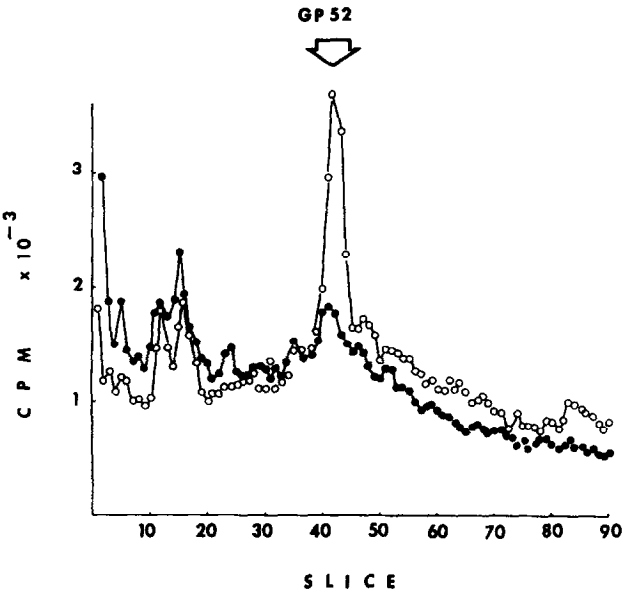

Figure 4
Effect of seeding density on the electrophoretic profile of radioiodinated cell surface proteins of BALB/cfC3H mouse mammary tumor cells: (A) 2 x 10^5 per cm^2 (●), and (B) 10^6 per cm^2 (○). Both cultures contained 10 µg/ml insulin and 5 µg/ml hydrocortisone. Volume of the extract prior to TCA precipitation was adjusted such that both contained equal protein content. The arrow indicates the position of gp 52. Reverse transcriptase activity in culture medium pooled just prior to iodination is as follows: (A) 3020 cpm, (B) 12,531 cpm. Background count of about 50 cpm has not been subtracted.

or very reduced amount. This is consistent with the observation that in our system the presence of hydrocortisone stimulates 5- to 10-fold the production of MTV into the culture medium (6,16,19). Figure 4 shows profiles obtained from cells seeded at two different densities. Cells cultured at a higher density revealed significantly more radioiodinatable gp 52 on their surfaces compared to the same cells seeded at a lower density. This is consistent with the previously reported observations (6,15) that the cultures in which cells were seeded at a higher density produce more MTV than those cultures in which cells were seeded at a lower density, even after correcting for cell number. These results suggest that there may be a correlation between abundance of radioiodinatable gp 52 on cell surface and production of MTV into the culture medium.

ACKNOWLEDGMENTS

We thank Drs. P. Bowman and J. Enami for critically reading the manuscript and Mr. John Underhill for photographic assistance. This investigation was supported by Grant Numbers CA05388 and CA 09041, awarded by the National Cancer Institute, DHEW.

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