Effect of Monensin on Mason-Pfizer Monkey Virus Glycoprotein Synthesis

S. CHATTERJEE, J. A. BRADAC, AND E. HUNTER*

Laboratory for Special Cancer Research, Department of Microbiology, The University of Alabama in Birmingham, Birmingham, Alabama 35294

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The effect of the monovalent carboxylic ionophore monensin on the biosynthesis, intracellular transport, and surface expression of the glycoproteins of Mason-Pfizer monkey virus was examined. Cells treated with monensin at concentrations of 10^{-7} or 10^{-6} M continued to synthesize virus particles, which from electron microscopic studies appeared to bud normally from the plasma membrane of the cells. However, the particles released had an altered buoyant density in sucrose gradients and were noninfectious. These noninfectious virions had a normal complement of non-glycosylated polypeptides but showed a significantly reduced amount of glycosylated proteins. The gp70 and gp20 polypeptides appeared to be completely absent, and a heterogeneous, higher-molecular-weight protein was observed on the virions instead. Studies on intracellular protein synthesis indicated that the precursor (Pr86^{env}) to gp70 and gp20 is synthesized normally but is not cleaved to the mature proteins. Immunofluorescence studies showed, however, that the uncleaved molecule is expressed on the cell surface. In this system, therefore, Mason-Pfizer monkey virus glycoprotein migration appears to occur in the presence of monensin, whereas the cleavage and insertion of the glycoproteins into virions are inhibited.

Mason-Pfizer monkey virus (M-PMV), a D-type retrovirus isolated from a spontaneous mammary carcinoma (3), has many characteristics in common with other known retroviruses (15, 19, 21) yet can be readily distinguished by its biological, biochemical, and immunological properties. This virus has two glycoproteins, gp20 and gp70, which are located on the external surface of the virion. Although it is known that these envelope glycoproteins are extremely important structural components, a limited amount of information is available about their biosynthesis, intracellular transport, and insertion into the surface membrane.

In the case of Rous sarcoma virus, the *env* gene mRNA is translated into a glycosylated 92,000-dalton precursor molecule (Pr92^{env}) (5, 9) on membrane-bound polysomes (18). The site in the cell where cleavage of the glycosylated precursor protein to the individual mature proteins, gp37 and gp85, occurs is not clear, although it has been reported that the precursor is cleaved intracellularly and that the cleavage products then migrate to the plasma membrane for virion assembly (9). Other workers, on the other hand, have found uncleaved precursor in newly synthesized virions; cleavage to gp85 and gp37, in this case, presumably occurred after release (8, 14). In the Friend murine leukemia virus only

gp70, the cleavage product of the glycosylated precursor, can be transported to the cell plasma membrane (27), whereas both gp70 and the precursor protein molecule are expressed at the surfaces of cells infected with mink cell focus-forming viruses (6). In this latter case, however, the precursor molecule is not incorporated into mature virions (6). Recent studies in this laboratory have shown that the two glycoproteins of M-PMV, gp70 and gp20, are synthesized as a glycosylated precursor of 86,000 daltons (Pr86^{env}) which is subsequently cleaved to the mature products (Bradac and Hunter, manuscript in preparation). Although the precursor appears to be highly glycosylated (with up to 12 carbohydrate side chains), the exact role of carbohydrate in its transport and maturation is not clear. Unlike those of the C-type retroviruses, the non-glycosylated structural proteins of M-PMV assemble in the cytoplasm to form intracytoplasmic A particles, which then migrate to the plasma membrane for virion assembly. The role of glycoproteins in this assembly process is unknown.

The monovalent carboxylic ionophore monensin has been shown to inhibit the transport and expression of membrane glycoproteins and several secretory proteins (17, 25, 26), and more recently it has been shown that monensin can

also block the migration of the membrane glycoproteins of certain enveloped viruses (1, 11, 12, 22). It therefore represents a useful tool for the analysis of viral glycoprotein synthesis and function.

In this communication, we report that monensin inhibits the cleavage of the glycosylated precursor Pr86^{env} into gp20 and gp70, but not the transport of this molecule to the cell surface. Furthermore, studies show that although the precursor molecule is expressed on the cell surface, it is incorporated at reduced levels in its uncleaved form into budding virions. The virions released from treated cells are noninfectious and have a significantly lower buoyant density than control virions.

MATERIALS AND METHODS

Virus and cell cultures. Normal human embryonic kidney (HEK) cells and the CMMT cell line (rhesus monkey mammary tumor cells cocultivated with rhesus monkey embryo cells) producing infectious M-PMV were kindly provided by M. Ahmed, Pfizer Inc., Maywood, N.J. C12, another source of M-PMV, was obtained by utilizing a modification of the microtiter protein A binding assay after infection of HEK cells with M-PMV. The detailed procedure will be published elsewhere (Chatterjee, Bradac, and Hunter, manuscript in preparation). Normal rhesus monkey fetal lung cells (DBS-FRHL-2) were purchased from Flow Laboratories, Inc., Rockville, Md.

DBS-FRHL-2 and CMMT cells were grown as described previously (2). HEK and C12 cells were grown in RPMI 1640 medium containing 10% heat-inactivated fetal calf serum, 5% tryptose phosphate broth, and 0.05% sodium bicarbonate.

Chemicals and radioisotopes. Reagents for polyacrylamide gel electrophoresis were purchased from Bio-Rad Laboratories, Richmond, Calif. Monensin was purchased from Calbiochem-Behring Corp., La Jolla, Calif. [³H]glucosamine (8.5 Ci/mmol), [¹⁴C]-amino acid mixture (50 mCi/mmol), and [³H]uridine (36 Ci/mmol) were obtained from ICN Pharmaceuticals Inc., Irvine, Calif. [³H]leucine (62 Ci/mmol) was purchased from Schwarz/Mann, Orangeburg, N.Y. Na¹²⁵I (15.5 mCi of ¹²⁵I per µg of iodine) was purchased from Amersham, Arlington Heights, Ill.

Assay for virus infectivity. The infectivity assay for M-PMV will be described in detail elsewhere (Hunter and Chatterjee, manuscript in preparation); a brief modified outline is presented here. Confluent monolayers of HEK cells grown in 96-well microtiter plates were inoculated with serial 10-fold dilutions of 24-h harvests collected from cells treated 48 h previously with 10⁻⁶ or 10⁻⁷ M monensin. Virus from untreated cultures of CMMT cells was inoculated as a control. After 1 h of adsorption, the cells were washed thoroughly with growth medium and incubated for 8 days at 37°C. Cells were then washed three times with Tris-buffered saline (pH 7.2) and fixed with a mixture of 95% ethyl alcohol and 5% glacial acetic acid at -20°C for 1 h. After fixation, cells were washed thoroughly with Tris-buffered saline containing 0.1% bovine serum albumin and 0.1% Triton X-100 and then incubated with goat anti-M-PMV p27 antiserum (25 µl per

well) for 1 h at room temperature. Cells were then washed three times with the same buffer, and approximately 5 × 10⁴ cpm of ¹²⁵I-labeled protein A (specific activity, 30 µCi/µg) from *Staphylococcus aureus* (Pharmacia Fine Chemicals, Uppsala, Sweden) was added to each well. Protein A iodination was carried out according to the procedure described by Greenwood et al. (7). After 30 min of incubation at room temperature, the cells were washed again thoroughly with the same buffer and air dried. The viral antigen-positive wells were detected by autoradiography for 24 h at -70°C on Kodak XR-2 film with a DuPont Cronex Hi-Plus intensifying screen. For quantitation of ¹²⁵I-protein A bound per well, the cells were disrupted with 1% sodium dodecyl sulfate (SDS) after autoradiography and counted in a Tracor model 1190 gamma counter (Tracor Analytic Inc., Atlanta, Ga.).

Electron microscopy. Monensin-treated and untreated cells grown in 60-mm dishes were washed thoroughly with phosphate-buffered saline (PBS) and fixed with 1% glutaraldehyde. Cells were postfixed with 1% osmium tetroxide and embedded in an epoxy resin mixture. Thin sections were stained with uranyl acetate and lead citrate as described previously (4). All the preparations were examined under a Philips EM 301 electron microscope.

Antisera. Monospecific antiserum against M-PMV gp70 was prepared in a New Zealand white rabbit by repeated intramuscular and subcutaneous injections of 250 µg of purified gp70 mixed with complete Freund adjuvant (1:1). gp70 was purified by affinity chromatography with Lentil-Lectin Sepharose 4B (Pharmacia Fine Chemicals), followed by SDS-polyacrylamide gel electrophoresis under reducing conditions. Protein was electrophoretically eluted from gel slices by using a model 1750 sample concentrator (ISCO, Lincoln, Nebr.). Serum was obtained 2 weeks after the third immunization and stored at -20°C. Monospecific antisera against M-PMV p27 and against Tween-ether-disrupted M-PMV were obtained from the Division of Cancer Cause and Prevention, National Cancer Institute, Bethesda, Md. Fluorescein-conjugated antiglobulins to rabbit immunoglobulin G (IgG) and goat IgG were obtained from Antibodies Incorporated, Davis, Calif. and from the Division of Cancer Cause and Prevention, National Cancer Institute, respectively.

Virus purification, preparation of radiolabeled virus, and polyacrylamide gel electrophoresis. To quantitate the amount of virions released from the monensin-treated and untreated cultures, CMMT cells were labeled with [³H]uridine (20 µCi/ml) in growth medium. Culture fluids were clarified and layered onto a 24 to 42% continuous sucrose gradient. After centrifugation at 40,000 rpm for 3 h in an SW41 rotor, the gradient fractions were collected, and sucrose density for each individual fraction was determined from the refractive index of the sucrose in each fraction. The amount of uridine-labeled virus at or near a density of 1.16 g/ml was also determined by counting a small sample (100 µl) from each gradient fraction.

To determine the effect of monensin on M-PMV polypeptide synthesis, CMMT cells were pretreated with monensin for 24 h and then labeled with [³H]glucosamine (200 µCi/ml) and a [¹⁴C]-amino acid mixture (20 µCi/ml) in the presence of monensin for 48 h. Control, untreated cultures were labeled in an identical manner. The culture fluids were clarified by low-

FIG. 1. Autoradiogram of ^{125}I -protein A binding assay for determination of the effect of monensin on the production of infectious M-PMV particles. Confluent monolayers of CMMT cells were treated with 0, 10^{-7} , and 10^{-6} M monensin for 48 h. Supernatant fluids were collected and clarified, and serial dilutions of these supernatant fluids were inoculated (50 μl per well), in duplicate, onto monolayers of HEK cells grown in a 96-well microtiter plate. After 1 h of adsorption, the cells were washed thoroughly, incubated at 37°C for 8 days, and then processed for the protein A binding assay as described in the text. Row C is the uninfected cell control.

speed centrifugation and layered onto a 24 to 48% (wt/wt) discontinuous sucrose gradient; the band at the 24 to 48% interphase was then recentrifuged on a 24 to 48% continuous sucrose gradient. The virus band was then collected, diluted, and pelleted by centrifugation at 45,000 rpm for 1 h. Virion polypeptides were separated on 12% discontinuous SDS-polyacrylamide slab gels as described (10). Radiolabeled virion polypeptides were located by slicing each lane into 1-mm-thick sections and counting in a toluene-Omnifluor scintillation fluid containing 10% Protosol (New England Nuclear Corp., Boston, Mass.). The molecular weights of viral polypeptides were determined by comparing the migration of these proteins with those of protein standards (Bio-Rad) electrophoresed in adjacent wells.

Radioimmunoprecipitation of intracellular polypeptides. Confluent monolayers of CMMT cells were thoroughly washed and incubated for 1 h in leucine-free Hams F10 medium in the presence or absence of monensin. The culture fluids were then replaced with 200 μl of the corresponding medium containing [^3H]leucine (1 mCi/ml), and the plates were incubated at 37°C for 10 min with occasional rocking. At the end of this time the radioactive medium was removed and replaced with 1 ml of ice-cold lysis buffer (1% Triton X-100, 1% sodium deoxycholate, 0.15 M NaCl, 0.05 M Tris, pH 7.5) in the case of pulse, or with warm complete growth medium (with or without monensin) in the case of pulse-chase. The medium was removed from chase plates at various times, and intracellular polypeptides were released by the addition of ice-cold lysis buffer. The lysates were centrifuged for 5 min in a Beckman Microfuge to remove nuclei. A volume of 100 μl of lysate and an appropriate amount of specific antiserum were incubated for 1 h at 37°C . After this incubation period, 100 μl of *S. aureus* immunoabsorbent was added. (A 10% suspension of Formalin-fixed, heat-killed Cowan 1 strain of *S. aureus* was prepared as described previously [13]). After an incubation

period of 30 min at room temperature, the immune complexes were washed with lysis buffer before being subjected to SDS-polyacrylamide gel electrophoresis as described above. Radioactive polypeptides were identified after impregnation of the gel with En 3 Hance (New England Nuclear Corp.) and drying, by the quantitative fluorographic procedure of Laskey and Mills (16).

Cell surface immunofluorescence. Monolayers of infected (C12) and uninfected (HEK) cells were treated with monensin for 48 h. Cells were then washed with PBS and treated with 0.05% EDTA in PBS for 5 to 10 min at room temperature. When most of the cells were rounded up, they were aspirated gently from the plate, centrifuged, washed thoroughly with PBS containing 0.1% bovine serum albumin, and resuspended in the same buffer. Portions of the cell suspension were then incubated with goat anti-M-PMV or rabbit anti-M-PMV gp70 at 0°C for 1 h with occasional shaking. Cells were then washed with PBS-bovine serum albumin buffer, and fluorescein-conjugated goat anti-rabbit IgG or fluorescein-conjugated rabbit anti-goat IgG was added. After incubation for 1 h at 0°C , the cells were finally washed thoroughly with PBS-bovine serum albumin buffer before being mounted on glass microscope slides in a glycerol-PBS solution (1:1). Cell surface antigens were observed by episcopic fluorescence, using a Nikon Fluorophot microscope.

RESULTS

Effect of monensin on the production of infectious virus particles. To determine the effect of monensin on the synthesis of infectious virions, CMMT cells were treated with 0, 10^{-6} , and 10^{-7} M monensin for two periods of 24 h, after which the supernatant fluids were assayed for infectious, replicating virus in a protein A-binding radioimmunoassay as described above. The results of this experiment (summarized in Fig. 1 and Table 1) clearly show that as little as 10^{-7} M monensin can block the production of infectious M-PMV by more than 99%. To confirm that this decrease in infectivity in the presence of monensin was not due to the failure of particle production, the following experiments were therefore performed.

TABLE 1. Effect of monensin on the synthesis of infectious M-PMV as determined by ^{125}I -protein A binding assay^a

Monensin concn (M)	^{125}I -protein A bound (cpm per well) at dilutions of:			% Inhibition ^b
	10^{-1}	10^{-2}	10^{-3}	
0	9,719	8,083	1,318	0
10^{-7}	141	52	0	>99
10^{-6}	63	2	0	>99

^a After the microtiter plate was processed for autoradiography (as described in the legend to Fig. 1), the cells were disrupted with 1% SDS and counted in a gamma counter for quantitation.

^b Percent inhibition was calculated from the data of counts per minute (cpm) at a 10^{-2} dilution.

Effect of monensin on the M-PMV particle production. Initial experiments were carried out to examine whether any physical particle formation occurs in the presence of monensin. Rhesus monkey (CMMT) cells producing M-PMV virus were treated with 0, 10^{-6} , and 10^{-7} M monensin for 48 h. Monensin-treated and untreated cells were then processed for electron microscopy as described above. Typical fields observed in the

electron microscopy are presented in Fig. 2. The presence of mature and budding virions can be seen in both the treated (Fig. 2B) and untreated (Fig. 2A) cells. Distinct M-PMV particle formation could also be observed in cells treated with the higher concentration of monensin (10^{-6} M) for 48 h (data not shown). These results indicate that monensin does not inhibit virus particle formation. A more direct biochemical study also

FIG. 2. Electron micrograph of thin sections of monensin-treated and untreated CMMT cells. (A) Untreated, control cells showing both mature and budding virions ($\times 44,000$). (B) Monensin (10^{-7} M)-treated cells also showing various stages of budding virions at the cell surface ($\times 24,000$). Cells were treated with monensin for 48 h before being fixed for electron microscopy. Distinct large intracytoplasmic dilated vesicles can be observed in the monensin-treated cells.

indicated that M-PMV particles are released in the presence of monensin. Monensin-treated (10^{-7} M) and untreated CMMT cells were labeled with [3 H]uridine for 36 h. The virus particles were then purified from the supernatant fluids by isopycnic sucrose gradients. A distinct virus peak was observed at a density of 1.16 g/ml when the culture fluid from the untreated cells was analyzed (Fig. 3). A similar virus band was also found in the case of supernatants from monensin-treated cells, although in this case the peak was shifted to a density of approximately 1.15 g/ml (Fig. 3). A similar shift in the density of virions was reported for Rous sarcoma virus when tunicamycin, a glycosylation inhibitor, or proteases were used to generate glycoprotein-free virions (20, 23). The amount of virus released in the presence of monensin was only reduced by approximately 30% compared to that produced in the absence of monensin. These results clearly demonstrate that in the presence of monensin near-normal amounts of M-PMV particles are synthesized, but that the majority of these particles are noninfectious.

Noninfectious M-PMV particles produced in the presence of monensin lack gp20 and gp70. To determine the polypeptide composition of the noninfectious M-PMV particles produced in the presence of monensin, CMMT cells were labeled with 14 C-amino acids and [3 H]glucosamine for 48 h in the presence of this ionophore (10^{-6} M). One set of CMMT cells was kept as an untreated control. The labeled virions were purified, and the polypeptides were separated by SDS-gel electrophoresis as described in Materials and Methods. Figure 4 shows the polypeptide pattern of virus particles synthesized in the presence and absence of monensin. Whereas the profile of the major non-glycosylated polypeptides p27, p14, p12, and p10 appeared to be the same in virions released from untreated or treated CMMT cells, a major difference could be observed in the two glycoprotein peaks. In untreated cells clear peaks of both carbohydrate and amino acid label could be seen in the position of gp70 and gp20. In monensin-treated cells (Fig. 4B), on the other hand, the gp20 peak was completely absent, and a broad heterogeneous peak with a somewhat slower migration rate replaced that of gp70. The specificity of the monensin inhibition for glycoproteins is confirmed by the lack of any effect on the non-glycosylated polypeptide pattern.

Monensin inhibits the cleavage of Pr86^{env}, the glycosylated precursor of gp20 and gp70. Since monensin has been shown to alter transport and secretion of cellular and viral polypeptides, it was of interest to determine the effect of monensin on the synthesis and processing of the viral proteins. Intracellular virus-coded proteins were

FIG. 3. Effect of monensin on the buoyant density of [3 H]uridine-labeled M-PMV particles. Virions released from CMMT cells 12 to 48 h after the addition of monensin were quantitated by sucrose density gradient centrifugation as described in the text. (O) Virions produced from untreated cells as control. (X) Virus particles synthesized from 10^{-7} M monensin-treated cells, showing a distinct shift in density. Sucrose densities were calculated from the refractive index of each fraction.

immunoprecipitated either with rabbit antiserum to the major virion glycoprotein, gp70, or with goat antiserum to the virus core polypeptide, p27. CMMT cell monolayers were pretreated with monensin for 1 h, pulse-labeled in the presence of monensin for 10 min with [3 H]leucine, lysed immediately or incubated for an additional 4 h in medium containing unlabeled leucine, and then lysed as described in Materials and Methods. Untreated CMMT cells were processed identically. Leucine-labeled polypeptides immunoprecipitated with the monospecific antisera were separated on 12% discontinuous polyacrylamide gels as shown in Fig. 5. In both treated and untreated cells the major polypeptide immunoprecipitated with anti-gp70 serum from pulse-labeled cells was Pr86^{env}, the glycosylated precursor to the two viral glycoproteins, gp70 and gp20. After the 4-h chase period, most of the radioactivity in the Pr86^{env} band was lost in the untreated control cells, and a diffuse band corresponding to one of its cleavage products, gp70, was observed (Fig. 5A, lane 4). In the monensin-treated cells, however, no band corresponding to gp70 could be seen after the chase period, although the intensity of the Pr86^{env} band was clearly reduced after a chase period.

FIG. 4. Effect of monensin on the synthesis of M-PMV structural proteins. (A) Polypeptide pattern of virions synthesized from the untreated CMMT cells and labeled with ^3H -glucosamine (X) and ^{14}C -amino acids (O), as analyzed by polyacrylamide slab gel electrophoresis. (B) Polypeptide profile of the radiolabeled virions released from the same cell line in the presence of 10^{-6} M monensin. See text for details.

Furthermore, there appeared to be an increase in the migration of this polypeptide after the 4-h chase period, suggesting that some incomplete processing of the carbohydrate moiety may have occurred. There was no subsequent appearance of any new polypeptide, indicating either that $\text{Pr}86^{\text{env}}$ was completely degraded or that it was incorporated into virions without first being cleaved. The presence of a small, glucosamine-labeled polypeptide peak in purified virions suggests that both processes may be occurring.

The major polypeptide immunoprecipitated with anti-p27 serum from pulse-labeled cells was $\text{Pr}78^{\text{gag}}$, the precursor to the non-glycosylated viral structural proteins. After the 4-h chase period, most of the radioactivity in the $\text{Pr}78^{\text{gag}}$ band was lost in the control cells, with the subsequent appearance of radiolabeled p27. In the monensin-treated cells, the cleavage of $\text{Pr}78^{\text{gag}}$ to yield p27 took place with essentially the same kinetics, although the process was reduced slightly.

FIG. 5. SDS-polyacrylamide gel electrophoresis of M-PMV specific proteins from untreated and monensin-treated cells: immunoprecipitation with antiserum directed against (A) M-PMV gp70 or (B) M-PMV p27. Replicate cultures of untreated CMMT cells (lanes 1 and 4) and cells pretreated for 1 h with either 10^{-7} M (lanes 2 and 5) or 10^{-6} M (lanes 3 and 6) monensin were pulse labeled for 10 min with [3 H]leucine in leucine-free Hams F10 medium. One culture was lysed immediately (lanes 1, 2, and 3). The others were rinsed and incubated in complete RPMI growth medium for 4 h (lanes 4, 5, and 6). Immunoprecipitates were analyzed by electrophoresis on 12% SDS-polyacrylamide gels. X in (A) denotes the faster-migrating form of the uncleaved precursor molecule, Pr86^{env}.

Migration of Pr86^{env} to the cell surface membrane in the presence of monensin. Experiments described in the previous section clearly showed that in the presence of monensin, cleavage of the glycosylated precursor, Pr86^{env}, does not occur. However, since limited amounts of glycosylated proteins were inserted into virions, it was of interest to determine whether this precursor molecule could be transported and expressed at high levels in the surface of the cell membrane. To determine the expression of Pr86^{env} at the cell membrane, infected C12 cells and uninfected

HEK cells were treated with 0 and 10^{-7} M monensin for 48 h with a change at 24 h to fresh monensin-containing medium. Unfixed cells were then processed for cell surface immunofluorescence by using anti-M-PMV and anti-M-PMV gp70 antisera as described in Materials and Methods. Figure 6 shows the result of such an experiment. It can be seen from this figure that in both the absence and presence of monensin, bright cell surface immunofluorescence could be observed. No background fluorescence was observed with uninfected cells. These results suggest that monensin does not significantly inhibit the transport to and expression of the M-PMV glycoprotein precursor molecule at the cell surface. The fact that the M-PMV-infected cells were treated with monensin for a prolonged period (48 h), during which time the cells were washed after 24 h, makes it unlikely that we are observing glycoprotein molecules that preexisted at the cell plasma membrane prior to monensin treatment. The 1,000-fold reduction in infectivity for virus released from such cells supports this conclusion. Furthermore, when rabbit anti-gp70 antiserum was used, in many instances the intensity of fluorescence on monensin-treated cells was higher than on untreated cells. Since this antiserum was raised against gp70 from an SDS-polyacrylamide gel, such a result may indicate the appearance of a modified, less native polypeptide at the cell surface.

DISCUSSION

The effect of the carboxylic ionophore monensin on the biosynthesis of M-PMV glycoproteins has been investigated. Studies on intracellular viral protein synthesis indicate that monensin has little effect on de novo protein synthesis, consistent with the report of Tartakoff and Vassalli (24). After a 10-min pulse of radioactive amino acid both Pr78^{gag}, the precursor to the non-glycosylated structural proteins, and Pr86^{env}, the glycoprotein precursor, had accumulated to the same extent in both treated and untreated cells. Whereas the gag precursor was cleaved in a 4-h chase period with approximately the same efficiency in the presence of monensin, the cleavage of Pr86^{env} was severely inhibited. The products of the latter precursor, gp70 and gp20, observed in untreated cells, were completely absent in those treated with the ionophore. Nevertheless, the intensity of the Pr86^{env} band decreased significantly during the chase period. Thus it appears that under the conditions of drug treatment, the precursor is either degraded intracellularly or shed from the surface of the cell. Immunofluorescence experiments using both anti-gp70 and anti-M-PMV antiserum indicated that glycoprotein antigens were present on the surface of unfixed, treated cells. The glyco-

FIG. 6. Cell surface immunofluorescence of M-PMV-infected HEK cells in the presence and absence of 10^{-7} M monensin. (A and B) Untreated and monensin-treated cells, respectively, stained with goat anti-M-PMV. (C and D) Untreated and monensin-treated cells, respectively, stained with rabbit anti-M-PMV gp70. Cells were treated with monensin for 48 h. Distinct surface immunofluorescence can be observed in both treated and untreated control cells.

protein of M-PMV differs from the G protein of vesicular stomatitis virus in this regard, since monensin blocks the transport of newly synthesized vesicular stomatitis virus glycoprotein from the Golgi body to the plasma membrane (1, 11). The product of the M-PMV *env* gene is also distinct from that of the Friend murine leukemia virus, in which the cleavage of the precursor is only reduced and transport to the plasma membrane is inhibited (22). The hemagglutinin of influenza virus, on the other hand, is transported

to the plasma membrane in a functional form (1), suggesting that more than one pathway might be involved in the transport of these different viral proteins. Little is known about these processes for M-PMV. For vesicular stomatitis virus it is known that the G protein is synthesized on membrane-bound polyribosomes, migrates to the Golgi system, and is then transported to the plasma membrane. Through the use of temperature-sensitive vesicular stomatitis virus mutants, it has been possible to identify stages at which

certain sugar and lipid residues are added (28). Such tools are currently not available for M-PMV.

Noninfectious virions are released from cells treated with either 10^{-7} or 10^{-6} M monensin. Since the viral glycoprotein does reach the plasma membrane, these experiments do not allow any conclusion to be made about the role of glycoprotein in the final stages of viral assembly, where the intracytoplasmic A-type particles are enveloped and released from the cell. The virions clearly do not contain any of the smaller glycoprotein, gp20, and gp70 appears to be replaced with a heterogeneous peak of less heavily glycosylated protein. In the absence of the cleavage products, it seems likely that this represents the inefficient insertion of a partially glycosylated uncleaved precursor into virus particles. The fact that the virions produced in the presence of the drug are 1,000-fold less infectious implies that this new glycoprotein is incapable of mediating viral attachment and penetration into the host cell. This differs from observations with influenza virus, in which the hemagglutinin protein produced in the presence of monensin is functional (1).

The noninfectious M-PMV virions produced in the presence of the drug have a lower buoyant density (1.15 g/ml) than virions produced by untreated cells (1.16 g/ml). Although the reason for this is unknown, it resembles the results obtained when Rous sarcoma virus is produced in the presence of tunicamycin (23). It is likely, therefore, that the change in density reflects a change in the carbohydrate content of the virus. Future experiments to determine the structure of the carbohydrate moieties present on these less dense virions may provide information in this regard.

It is clear that for different viruses monensin has a variety of inhibitory effects. How much this depends on the primary structure of the viral glycoprotein and how much depends on the type of host cell is not understood. Nevertheless it is likely that such inhibitors will ultimately provide significant information on the biosynthesis and transport of these structurally important proteins.

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