JOURNAL OF VIROLOGY, Nov. 1979, p. 412-419
0022-538X/79/11-0412/08$02.00/0

Vol. 32, No. 2

Inhibition of Rous Sarcoma Virus Replication by 2-Deoxyglucose and Tunicamycin: Identification of an Unglycosylated *env* Gene Product

ROBERT STOHRER AND ERIC HUNTER*

Department of Microbiology, The Medical Center, University of Alabama in Birmingham, Birmingham, Alabama 35294

Received for publication 15 May 1979

Two inhibitors of glycosylation, 2-deoxyglucose and tunicamycin, depressed the synthesis of infectious Rous sarcoma virus greater than 100-fold. Under the same conditions only a two- to threefold decrease in the production of virus particles was observed. The noninfectious particles had a lower density (1.145 g/ml) in isopycnic sucrose gradients and lacked the two virion glycoproteins, gp85 and gp37, found on infectious virions. The four internal structural proteins of the virus, p27, p19, p15, and p12, appeared to be assembled normally into the noninfectious virus. Polypeptides related to the Rous sarcoma virus glycoproteins were immunoprecipitated from pulse-labeled Rous sarcoma virus (Prague strain, subgroup B)-transformed cells. $pr95^{gp}$, the polyprotein precursor to gp85 and gp37, was the major protein precipitated from untreated cells. This was absent in both tunicamycin- and 2-deoxyglucose-treated cells, and a new polypeptide of molecular weight 57,000 to 58,000 was the major species precipitated. In tunicamycin-treated cells this product was unstable and was degraded during a 2-h chase; in 2-deoxyglucose-treated cells, on the other hand, the polypeptide appeared to be more stable and underwent partial glycosylation. The synthesis and processing of pr76, the polyprotein precursor to the internal structural proteins of the virion, occurred normally in both treated and untreated cells. It is concluded that the unglycosylated *env* gene product is a polypeptide of molecular weight 57,000 to 58,000.

Rous sarcoma virus (RSV) is an enveloped RNA virus which derives its lipid bilayer from the plasma membrane of the host cell. Inserted into the exterior surface of the viral membrane are protein spikes consisting of two glycoproteins, gp85 and gp37, that are responsible for attachment and penetration of the virus into a susceptible host cell (23, 32). In common with other enveloped viruses, the production of infectious RSV can be abrogated by inhibitors of glycosylation (9, 19, 29). In previous studies we demonstrated that high levels of glucosamine not only prevented the production of infectious virus, but also inhibited virus particle production. The nature of this inhibition was complex and resulted from secondary effects of the high levels of glucosamine on cellular metabolism in addition to an inhibition of glycosylation of viral polypeptides (7). In contrast, treatment of murine leukemia virus-producing cells with 2-deoxyglucose (2-DG), an analog of mannose, resulted in the synthesis of noninfectious virions (22). In addition, treatment of RSV-producing cultures with the potent inhibitor of glycosylation tunicamycin (TM) has been reported to result in the synthesis of virions lacking gp85 and gp37 (29).

Treatment of influenza virus-infected cells with 2-DG or TM results in the synthesis of a partially glycosylated or unglycosylated hemagglutinin polypeptide, HA_0 (21, 29). Similar observations have been made with Sindbis virus (18), Semliki Forest virus (12, 29), and vesicular stomatitis virus (5), where intracellular, partially glycosylated or nonglycosylated polypeptides have been identified. Initial attempts to find a similar polypeptide in TM-treated, RSV-infected cells were unsuccessful (29).

In this paper we report that both 2-DG and TM severely inhibit the synthesis of infectious RSV without significantly affecting the synthesis of RSV virions. These noninfectious virions lack both gp85 and gp37. In the presence of either inhibitor, $pr95^{gp}$, the partially glycosylated precursor to the two glycoproteins (4, 6, 16, 20, 24, 30), is no longer synthesized, and a new polypeptide of molecular weight 57,000 to 58,000 has been identified which is specifically recognized by antisera to gp85 and gp37.

MATERIALS AND METHODS

Tissue culture. Fertile chicken eggs of the C/E phenotype were purchased from SPAFAS Inc. Primary and secondary chicken embryo fibroblast cultures were prepared as described previously (8) and were tested for chick helper factor expression before use (34). Only cells derived from chick helper factor-negative embryos were used in these studies.

Subgroup B virus of the Prague strain of RSV (PR-B), cloned twice on chick helper factor-negative cells, was used in all the experiments. Cells were infected at a multiplicity of infection of 0.1 focus-forming unit/cell in Hams F10 medium supplemented with 10% tryptose phosphate broth, 5% calf serum (Biocell Inc., Venice, Calif.), and, to enhance adsorption, 2 μg of Polybrene per ml. Duplicate cultures of confluently transformed cells were treated as described in the text. Focus assays for infectious transforming virus were carried out as described previously (8).

Chemicals and radioisotopes. TM was a generous gift from R. L. Hamill, Lilly Research Laboratories, Indianapolis, Ind. 2-DG was purchased from Sigma Chemical Co., St. Louis, Mo. Reagents for gel electrophoresis were obtained from Bio-Rad Inc., Richmond, Calif. [^{3}H]leucine (Schwarz/Mann, Orangeburg, N.Y.; 65 Ci/mmol), ^{14}C-labeled amino acid (ICN, Irvine, Calif.; 50 mCi/mmol), and [^{3}H]glucosamine (ICN; 6.3 Ci/mmol) were lyophilized before use and redissolved in the appropriate culture medium.

Preparation of radiolabeled virus. To quantitate the synthesis of virions by treated and untreated cells, transformed cultures were labeled for 16 h with [^{3}H]uridine (10 μCi/ml) in complete growth medium. Culture fluids were clarified and layered onto a 24 to 48% continuous sucrose gradient. After centrifugation for 2.5 h at 40,000 rpm in an SW41 rotor, the gradients were unloaded from the bottom and the amount of uridine-labeled virus banding at or near a density of 1.16 g/ml was determined. The gradient of sucrose densities for each individual tube was determined from the refractive index of the sucrose in each fraction.

In studies to determine the effect of inhibitors on the virion polypeptide profile, transformed cell cultures were preincubated for 5 h in 2-DG or TM and then labeled for 16 to 20 h in complete growth medium containing 120 μCi of [^{3}H]glucosamine per ml and 16 μCi of ^{14}C-amino acids per ml. The culture fluids were clarified by low-speed centrifugation (2,000 rpm, IEC refrigerated centrifuge) and layered onto a discontinuous sucrose (24%, wt/wt)-potassium tartrate (40%, wt/wt) gradient as described previously (10). Virus banding at the sucrose-tartrate interface was collected, diluted in STE (saline-Tris-EDTA [10]) buffer, and pelletted by centrifugation at 50,000 rpm for 1 h in an SW50.1 rotor.

Antisera and immunoadsorbents. Rabbit antisera to avian sarcoma virus virions and the major structural polypeptide, p27, of RSV were prepared by multiple inoculation of 250 μg of protein into New Zealand white rabbits as described previously (10). Rabbit antiserum to lectin-purified RSV-PR-B glycoproteins was kindly provided by Mike Hayman.

A 10% (wt/vol) suspension of Formalin-fixed, heat-killed *Staphylococcus aureus* (Cowan 1 strain), prepared as described previously (2, 15), was used as an immunoadsorbent for antibody-antigen complexes.

Immunoprecipitation of intracellular polypeptides. For pulse-chase experiments confluently transformed cells seeded in 35-mm culture dishes were washed and incubated for 1 h in leucine-free Hams F10 nutrient medium containing the desired concentration of inhibitor. The culture fluids were then replaced with 200 μl of the same medium containing [^{3}H]leucine (1 mCi/ml), and the plates were incubated at 37°C for 15 min with frequent rocking. At the end of this time the radioactive medium was removed and replaced with 1 ml of ice-cold lysis buffer (1) (in the case of a pulse plate) or with warm complete growth medium (in the case of a pulse-chase plate).

The medium was removed from chase plates at various times, and intracellular polypeptides were released by addition of ice-cold lysis buffer. The lysates were sonicated briefly to shear any released DNA and then centrifuged for 5 min in a Beckman Microfuge. A volume of 100 μl of lysate and sufficient antiserum to precipitate all viral proteins present were incubated for 1 h at 37°C, and then 100 μl of *S. aureus* immunoadsorbent was added. After a further 30 min at room temperature, the immune complexes were washed twice with lysis buffer and once with 20 mM Tris-hydrochloride (pH 8.0) before dissociation in gel buffer.

Polyacrylamide gels. Purified virion polypeptides and immunoprecipitated proteins were separated on discontinuous slab gels as described previously (10). Acrylamide concentrations of 10, 12, and 5 to 20% gradients were used as described in the figure legends. Radiolabeled polypeptides were localized after Coomassie brilliant blue staining either by slicing each lane into 1-mm-thick sections and counting in a toluene-Omnifluor scintillation fluid containing 10% Protosol or by the quantitative fluorographic procedure of Laskey and Mills (17).

The molecular weights of viral polypeptides were determined by comparing the migration of these proteins with that of protein standards electrophoresed in adjacent wells. The protein standards used in these experiments were myosin (200 kilodaltons [kd]), β-galactosidase (116.5 kd), phosphorylase B (94 kd), bovine serum albumin (68 kd), ovalbumin (43 kd), carbonic anhydrase (30 kd), soybean trypsin inhibitor (21 kd), and lysozyme (14.3 kd) (Bio-Rad Inc.).

RESULTS

Inhibition of infectious RSV production by 2-DG and TM. Initial experiments were performed to determine the effect of different concentrations of 2-DG and TM on the replication of infectious RSV. Confluently transformed monolayers of chicken embryo cells were treated with 0, 5, 10, and 20 mM 2-DG or 0, 0.25, 0.5, 1.0, and 2.0 μg of TM per ml for a total of 12 h. Cell monolayers were washed at 7 and 8 h after initiation of treatment, and virus produced between 8 and 12 h was harvested for infectivity assays. The results of such an experiment are

TABLE 1. *Effect of glycosylation inhibitors on synthesis of infectious RSV*

Inhibitor	Virus titer (FFU/ml)[a]	Efficiency of replication[b]
2-DG (mM)		
0	1.3×10^6	
5	3.4×10^3	0.003
10	3.3×10^3	0.003
20	3.0×10^3	0.002
TM (μg/ml)		
0	1.3×10^6	
0.25	1.1×10^4	0.008
0.5	7.4×10^3	0.006
1.0	8.2×10^3	0.006
2.0	4.8×10^3	0.004

[a] Inhibitors were added to cell monolayers confluently transformed with RSV-PR-B. Cells were washed twice at 7 and 8 h posttreatment; then virus released in the 8- to 12-h period was harvested and assayed in a focus assay to determine the titer of the virus. FFU, Focus-forming units.

[b] Efficiency of replication = titer of virus produced in presence of inhibitor/titer of virus produced in absence of inhibitor.

summarized in Table 1. All concentrations of 2-DG reduced the production of infectious virus more than 300-fold. As little as 0.25 μg of TM per ml inhibited synthesis of focus-forming virus greater than 99% and addition of 2 μg resulted in almost a 300-fold reduction. In the case of 5 and 10 mM 2-DG infections, virus production recovered to within 20% that of untreated cultures within 12 h of removal of the inhibitor, indicating that inhibition was not merely the result of a toxic effect on the cells. Infectious virus production in cultures treated with as little as 0.25 μg of TM per ml continued to decline during the 12 h after removal of the drug even though the cells showed no apparent toxic effects (data not shown). Washing of the monolayers at 7 and 8 h after initiation of treatment was found to be essential for accurately assessing the effects of 2-DG and TM on infectious virus synthesis. In the absence of these washes low levels of inhibition (5- to 10-fold) were observed, which may reflect the delayed release of previously synthesized focus-forming virus. This may explain the poor inhibition observed by Schwarz et al. (29) after 8 h of treatment of RSV-infected cells with TM.

Effect of glycosylation inhibitors on virion synthesis. Previous studies on the inhibition of RSV glycoprotein synthesis by glucosamine showed that virus particle production was completely abrogated (7, 9). The effect of 2-DG and TM on virion production was therefore investigated. Confluently transformed cells were treated for 4 h with either 10 mM 2-DG or 1 μg of TM per ml, and then the medium was replaced with medium containing 100 μCi of [^{3}H]uridine per ml in addition to the inhibitors. Virus produced during the next 16 h was identified as a peak of uridine label in isopycnic sucrose gradients. The results of such an experiment are shown in Fig. 1. A peak of radioactivity at a density of 1.16 g/ml was observed after centrifugation of untreated supernatants (Fig. 1A). Smaller but significant peaks of uridine-labeled virus were observed in the supernatants of treated cells. In both cases, however, the virus peak had shifted to a less dense region of the gradient, such that virions produced in the presence of 1 μg of TM banded at a density of 1.145 to 1.15 g/ml. This shift in density is similar to that described by Rifkin and Compans (23) after the removal of the external glycoproteins of RSV with proteases.

The two- to threefold reduction in total amount of uridine-labeled virions produced in the presence of 2-DG (32% of control) or TM (52% of control) in these experiments was confirmed by quantitative competition radioimmunoassays (data not shown). A comparison of the reduction in infectious virus (100- to 200-fold) with that of virions (2- to 3-fold), in the presence

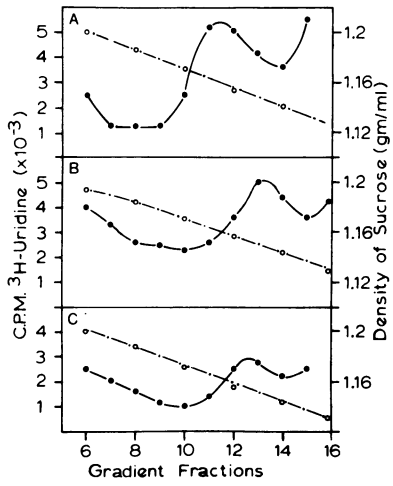

FIG. 1. *[^{3}H]uridine-labeled virus synthesized in presence of glycosylation inhibitors. Chicken embryo cultures, confluently infected with RSV-PR-B, were untreated as controls (A) or were treated with 1 μg of TM per ml (B) or 10 mM 2-DG (C). [^{3}H]uridine (10 μCi/ml) was added after 4 h and all culture fluids were harvested 16 h later. After clarification in a low-speed centrifuge, the supernatants were loaded onto a continuous 24 to 48% sucrose gradient and processed as described in the text. The density of sucrose in each fraction was calculated from the refractive index of that fraction.*

of these glycosylation inhibitors indicates that a significant number of noninfectious virions are synthesized. The nature of these virus particles was investigated (see below).

Noninfectious virions lack gp85 and gp37. To determine the polypeptide composition of the noninfectious virions synthesized in the presence of 2-DG and TM, infected cells were labeled with ^{14}C-amino acids and [^{3}H]glucosamine. Double-labeled virions were purified as described in Materials and Methods, and the virion-associated polypeptides were resolved in 12% sodium dodecyl sulfate-polyacrylamide gels. The results of such an experiment are shown in Fig. 2 and 3. The polypeptide profile of virions synthesized by untreated cells (Fig. 2A and 3A) is characteristic of RSV, with two glucosamine-labeled glycoproteins, gp85 and gp37, in addition to the non-glycosylated peaks of p27, p19, p15, and p12. In contrast, virions synthesized in the presence of either 2-DG (Fig. 2B) or TM (Fig. 3B) lacked both glycoprotein peaks, despite the fact that the internal structural protein peaks appear to be comparable to those of untreated virions. Furthermore, no new polypeptides could be observed in the noninfectious virus, indicating that if a non-glycosylated polypeptide is synthesized in treated cells, it is not associated with the virus particle at a detectable level.

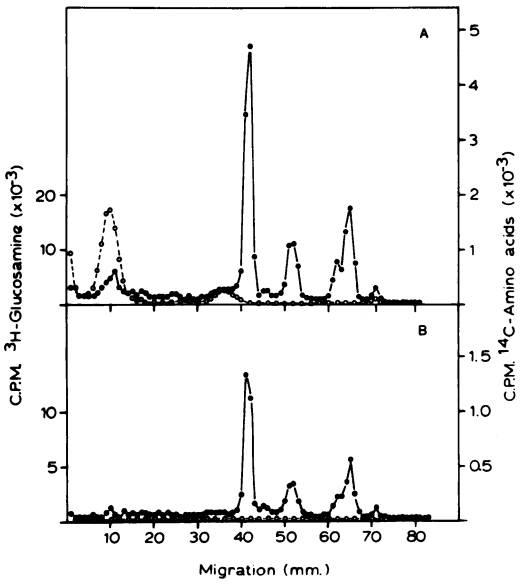

FIG. 2. *Virions synthesized in the presence of 10 mM 2-DG lack gp85 and gp37. Control infected cells (A) and infected cells treated for 4 h with 10 mM 2-DG (B) were labeled for a further 16 h with [^{3}H]-glucosamine* (○) *and ^{14}C-amino acids* (●). *Virus released in this period was purified, and the labeled polypeptides were separated as described in the text.*

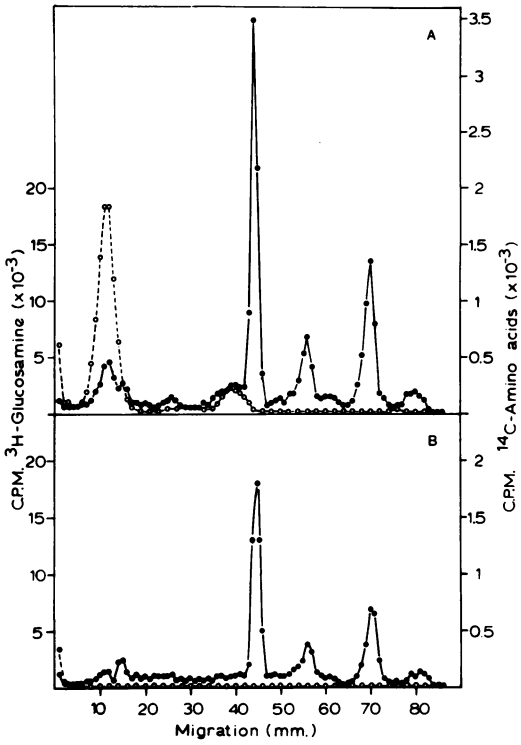

FIG. 3. *Virions synthesized in the presence of 2 μg of TM per ml lack gp85 and gp37. Control infected cells (A) and infected cells treated for 4 h with 2 μg of TM per ml (B) were labeled and processed as described in the legend to Fig. 2.*

A non-glycosylated polypeptide of 57 to 58,000 kd is synthesized in 2-DG- or TM-treated cells. Inhibition of glycosylation in influenza virus-infected cells results in the synthesis of a non-glycosylated hemagglutinin, HA_0 (21, 28). To determine whether a similar polypeptide was synthesized in RSV-infected cells treated with these inhibitors, intracellular virus-coded proteins were immunoprecipitated with rabbit antisera to the virion glycoproteins or to the virus core polypeptide p27. Cells, uniformly transformed by RSV-PR-B, were pulsed for 15 min with [^{3}H]leucine, lysed immediately or incubated for 2 h more in medium containing excess unlabeled leucine, and then lysed as described in Materials and Methods. Leucine-labeled polypeptides immunoprecipitated by rabbit antibody to RSV glycoproteins were separated on 5 to 20% gradient polyacrylamide gels as shown in Fig. 4A. The major polypeptide immunoprecipitated from pulse-labeled, untreated cells was pr95^{gp}, the incompletely glycosylated precursor to gp85 and gp37. A minor band of pr76, the precursor polyprotein to the internal structural proteins of RSV, is also ob-

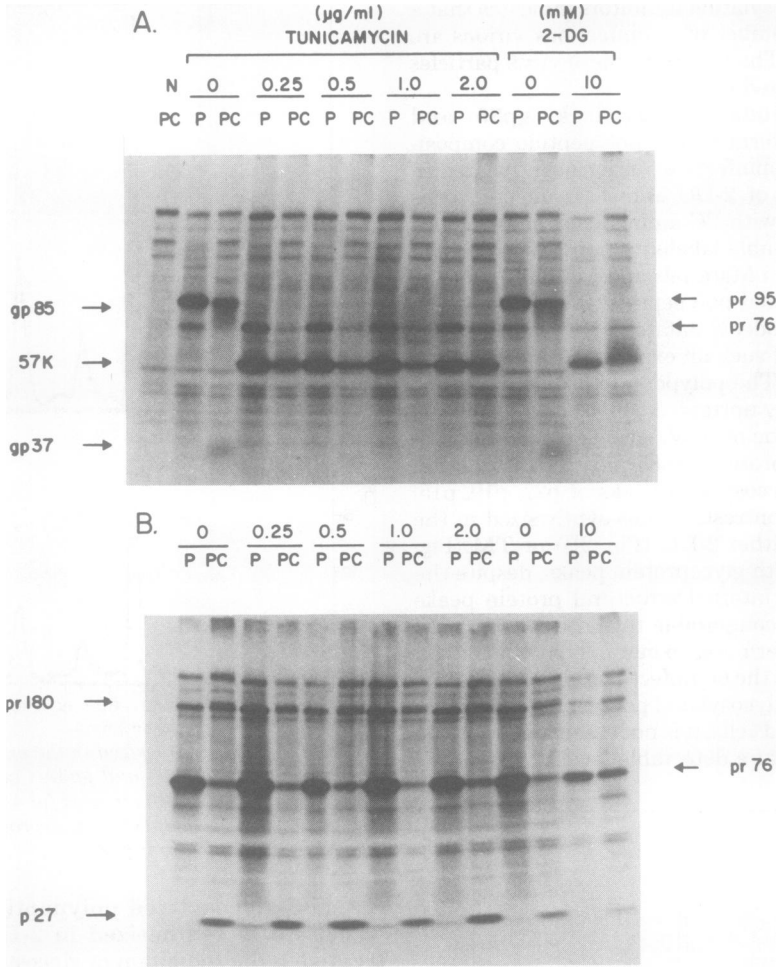

FIG. 4. *Autoradiogram of a 5 to 20% polyacrylamide slab gel resolving [^{3}H]leucine-labeled proteins immunoprecipitated from RSV-infected cells treated with 2-DG and TM. Duplicate cultures of infected cells were treated for 4 h with each of the various concentrations of inhibitors shown; the medium was then removed and replaced with leucine-deficient medium containing the same level of inhibitors. After a further incubation for 1 h the medium was replaced with 0.20 ml of the same medium containing [^{3}H]leucine (1 mCi/ml), and the cells were pulse-labeled for 15 min. One plate of each pair was lysed immediately (pulse [P]) to the other, complete growth medium was added, and incubation was continued for a further 120 min (pulse-chase [PC]), when it was also lysed as described in the text. Lysates were immunoprecipitated as described in the text with antisera to the virion glycoproteins (A) or the major structural protein of the virion, p27 (B). A control plate of uninfected, untreated chicken embryo cells was labeled and chased in an identical manner and is shown in lane N, PC.*

served due to the presence of a low level of contaminating antibody to one of these components. The antigenic determinant recognized on pr76 is type specific, since after immunoprecipitation of cells infected with viruses of either subgroup A or C, a pr95^{gp} polypeptide band is observed in the absence of a pr76 band (data not shown).

After the 2-h chase period much of the radioactivity in the pr95^{gp} band is lost, and diffuse bands corresponding to its cleavage products, gp85 and gp37, are observed.

Cells pulse-labeled in the presence of TM or 2-DG lack a major polypeptide band at 95 kd. Instead, a new major polypeptide band is observed with a molecular weight of 57,000 in TM-treated cells and 58,000 in 2-DG-infected cells. This polypeptide appears to be lost from TM-treated cells during the 2-h chase (Fig. 4A, PC tracks), without the appearance of any new pol-

ypeptides, indicating that the non-glycosylated polypeptide is turned over. On the other hand, the similar sized product found in 2-DG-treated cells increases in molecular weight in the 2-h chase period, producing a heterogeneous protein band (Fig. 4A, 10 mM 2-DG, PC track). This may indicate that partial glycosylation of the 58-kd product can occur. The 57- to 58-kd polypeptide is immunoprecipitated only by antiserum directed against the RSV glycoproteins and is not observed when either treated or untreated cells are immunoprecipitated with antibody to the major structural protein, p27, of RSV (Fig. 4B). In this case the major polypeptide observed after a 15-min pulse is pr76, and during the 2-h chase the intensity of this band is significantly reduced, with the concomitant appearance of a band corresponding to p27. Neither glycosylation inhibitor appears, therefore, to interfere with the synthesis or processing of the precursor to the internal structural proteins of RSV.

DISCUSSION

This is good evidence that the envelope glycoproteins, gp85 and gp37, of RSV are required for infectivity of these viruses (3, 13, 23, 31). Indeed, the antigenically different glycoproteins of different virus subgroups (11, 21, 32) determine the host range of any particular virus. The severe reduction we observed in the synthesis of infectious RSV in cells treated with either inhibitor of glycosylation is consistent with this concept, since both 2-DG and TM might be expected to prevent the production of the glycosylated forms of gp85 and gp37. In a previous study we showed that high levels of glucosamine could inhibit RSV replication; however, this amino sugar has profound effects on other aspects of cellular metabolism in addition to interfering with glycosylation (14, 27). In addition, treatment of RSV-infected cells with glucosamine was previously shown to block both infectious virus and virion synthesis (7, 9). This is in contrast to the results reported here for both 2-DG and TM and may reflect the complex nature of glucosamine inhibition. Despite the fact that infectious virus synthesis is inhibited by more than 99.0% at even the lowest concentrations of 2-DG or TM used in these experiments, virion synthesis was reduced only twofold in the presence of 1 μg of TM per ml or threefold in 10 mM 2-DG (Fig. 1). This observation is consistent with that reported for the PR-C strain of RSV in studies with TM (29). The small reduction in virion production may reflect the slightly depressed rate of protein synthesis observed in 2-DG- or TM-treated cells (data not shown).

The high level of noninfectious virus synthesis in the presence of either 2-DG or TM is in contrast to that observed with fowl plague virus (29), Semliki Forest virus (29), Sindbis virus (18), and the San Juan strain of vesicular stomatitis virus (5), where virion synthesis is either completely abolished or reduced greater than 90%.

In these viruses it appears that the virion glycosylated proteins or a glycosylated cellular protein is necessary for virus assembly. RSV, on the other hand, appears capable of assembling virions at high efficiency in the absence of glycosylation. Furthermore, these noninfectious virions appear to lack any detectable gp85 and gp37, although they contain normal proportions of the internal structural polypeptides p27, p19, p15, and p12. The noninfectious virions, produced in the presence of 2-DG or TM, therefore resemble RSV(−) or SR-NY8 virions in which genetic lesions prevent the synthesis of glycoproteins (13, 24, 33).

The WSN strain of influenza virus is similar to RSV in the synthesis of virus particles under conditions where glycosylation is blocked. Nakamura and Compans (21) found that virions lacking both hemagglutinin and neuraminidase, or their non-glycosylated counterparts, were produced from WSN-infected MDBK cells treated with TM. In the presence of 10 mM 2-DG, however, WSN virion synthesis was reduced 30- to 40-fold. In contrast, we have observed essentially the same level of virion synthesis with both 10 mM 2-DG and 2 μg of TM per ml. If, however, the concentration of 2-DG was increased from 10 to 20 mM, a significant reduction (>20-fold) in virion synthesis was observed. A similar reduction was observed at low levels of 2-DG (0.25 mM) if pyruvate was substituted for glucose as a source of carbon in the culture medium (unpublished data).

It is of interest that the virions synthesized in the presence of either 2-DG or TM possessed a significantly lower density than virions from untreated cells. Similar observations have been made by Schwarz et al. (29) in their studies on RSV and by Nakamura and Compans (21) for influenza virus made in the presence of TM. The reduction in virion density (0.01 to 0.015 g/ml) we have observed is essentially the same as that seen after removal of the virion glycoproteins by protease (23).

The two virion glycoproteins of RSV are derived from a single polyprotein precursor molecule, $pr95^{gp}$, (4, 6, 16, 20, 24, 30). This partially glycosylated molecule is the major virus glycoprotein observed in pulse-labeled cells after immunoprecipitation with suitable anti-glycoprotein or anti-whole virus antisera (Fig. 4). In the presence of both 2-DG (10 mM) and TM (0.25

to 2.0 μg/ml) a new polypeptide of molecular weight 57,000 to 58,000 is specifically precipitated by antiserum to the virion glycoproteins from cells labeled in a 15-min pulse of [^{3}H]leucine. No radioactivity is observed in the region of pr95^{gp} and no other new virus-specific bands are observed, which suggests that the 57- to 58-kd protein is the non-glycosylated polypeptide backbone of the pr95^{gp}. Furthermore, only the 57- to 58-kd polypeptide was observed in 2-DG- or TM-treated cells pulsed for short periods (2 min) or treated with the protease inhibitors tolylsulfonyl phenylalanyl chloromethyl ketone (100 μg/ml), tolylsulfonyl-L-lysine chloromethyl ketone (100 μg/ml), and phenylmethylsulfonyl fluoride (250 μg/ml) (unpublished data). The fact that the polypeptide is immunoprecipitated from both 2-DG- and TM-treated, PR-B-infected cells but not from uninfected cells and that it is specifically precipitated by anti-glycoprotein antiserum and not anti-p27 antiserum is strong evidence that this is indeed the non-glycosylated translation product of the *env* gene.

During a 120-min chase period the radioactivity associated with the pr95^{gp} band in untreated cells is reduced (Fig. 4, 0 TM, PC), and diffuse bands corresponding to its completely glycosylated cleavage products, gp85 and gp37, appear. Different effects of a 120-min chase period are observed in TM- and 2-DG-treated cells. At all levels of TM a significant reduction in the radioactivity associated with the intracellular 57-kd band is observed in the chase, with no apparent appearance of new products. Since this unglycosylated polypeptide is not observed after a chase in virions synthesized in the presence of TM (Fig. 3B), it appears that the disappearance of the band is due to degradation by cellular proteases. Unglycosylated forms of the fowl plague virus hemagglutinin and WSN hemagglutinin also appear to be highly susceptible to degradation by proteases, such that HA_0 of fowl plague virus can only be identified in the presence of the protease inhibitor tolylsulfonyl-L-lysine chloromethyl ketone (29).

The 58-kd polypeptide found in 2-DG-treated cells, on the other hand, appears to be more stable, and after the 120-min chase a diffuse band with increased molecular weight (58,000 to 75,000) in addition to the 58-kd protein is observed in immunoprecipitates with anti-glycoprotein antiserum. We have interpreted this to mean that additional sugars are being added to the polypeptide during the chase period. 2-DG probably inhibits glycosylation at the level of the sugar transferases, where it could act as a competitive inhibitor for mannose (26). Since 2-deoxy-2-fluoro-mannose inhibits glycosylation without being incorporated into the acceptor proteins (25), the sugar analogs may block transfer of mannose to the (*N*-acetylglucosamine)$_2$-dolichol intermediate. The latter might, however, still transfer to the protein its two *N*-acetylglucosamine residues to which additional sugars might be added during the 2-h chase period. The intracellular location of the polypeptides observed after 2-DG and TM treatment both in the pulse and after the chase remains to be determined.

A question still remains as to why Schwarz et al. (29) were unable to identify an unglycosylated polypeptide in their studies. It is possible that the 57- to 58-kd polypeptide lacks any type-specific determinants and can be recognized only by antibodies directed against group-specific determinants. The antiserum used in this study was raised against a lectin-purified gp85-gp37 complex and recognizes predominantly group-specific determinants (M. Hayman, personal communication). Antisera raised against whole RSV virions precipitate pr95^{gp} efficiently but precipitate the 57- to 58-kd protein less efficiently (unpublished data), which may reflect the titer of group-specific antibodies in the serum.

The conclusion that the 57- to 58-kd polypeptide represents a non-glycosylated translation product of the *env* gene would be further supported by the identification of an identical polypeptide from the in vitro translation of 28S mRNA from virus-infected cells or 28S fragmented viral RNA; such studies are currently in progress.

ACKNOWLEDGMENTS

This work was supported by grant VC-215A from the American Cancer Society.

We thank Jack Hill for expert technical assistance.

LITERATURE CITED

1. **Brugge, J. S., and R. L. Erikson.** 1977. Identification of a transformation-specific antigen induced by avian sarcoma virus. Nature (London) **269**:346–348.
2. **Cullen, S. E., and B. D. Schwartz.** 1978. An improved method for isolation of H-2 and Ia alloantigens with immunoprecipitation induced by protein A-bearing staphylococci. J. Immunol. **117**:136–142.
3. **Duesberg, P. H., G. S. Martin, and P. K. Vogt.** 1970. Glycoprotein components of avian and murine RNA tumor viruses. Virology **41**:631–646.
4. **England, J. M., D. P. Bolognesi, B. Dietzschold, and M. S. Halpern.** 1977. Evidence that a precur r glycoprotein is cleaved to yield the major glyc· pro'ein of avian tumor virus. J. Virol. **21**:810–814.
5. **Gibson, R., R. Leavitt, S. Kornfeld, and S. Schlesinger.** 1978. Synthesis and infectivity of vesicular stomatitis virus containing nonglycosylated G protein. Cell **13**:671–679.
6. **Hayman, M. J.** 1978. Synthesis and processing of avian sarcoma virus glycoproteins. Virology **85**:475–486.
7. **Hayman, M. J., E. Hunter, and P. K. Vogt.** 1976.

Inhibition of avian sarcoma virus replication by glucosamine: a specific effect on the synthesis and processing of viral proteins. Virology **71**:402–411.

8. **Hunter, E.** 1979. Biological techniques for avian sarcoma viruses. Methods Enzymol. **58**:379–393.
9. **Hunter, E., R. R. Friis, and P. K. Vogt.** 1974. Inhibition of avian sarcoma virus replication by glucosamine. Virology **58**:449–456.
10. **Hunter, E., M. J. Hayman, R. W. Rongey, and P. K. Vogt.** 1976. An avian sarcoma virus mutant which is temperature sensitive for virion assembly. Virology **69**: 35–49.
11. **Ishizaki, R., and P. K. Vogt.** 1966. Immunological relationships among envelope antigens of avian tumor viruses. Virology **30**:375–387.
12. **Kaluza, G., M. F. G. Schmidt, and C. Scholtissek.** 1973. Effect of 2-deoxy-D-glucose on the multiplication of Semliki forest virus and the reversal of the block by mannose. Virology **54**:179–189.
13. **Kawai, S., and H. Hanafusa.** 1973. Isolation of a defective mutant of avian sarcoma virus. Proc. Natl. Acad. Sci. U.S.A. **70**:3493–3496.
14. **Keppler, D., J. Rudigier, E. Bischoff, and K. Decker.** 1970. The trapping of uridine phosphates by D-galactosamine, D-glucosamine, and 2-deoxy-D-galactose. A study on the mechanism of galactosamine hepatitis. Eur. J. Biochem. **17**:246–253.
15. **Kessler, S. W.** 1975. Rapid isolation of antigens from cells with a staphylococcal protein A-antibody adsorbent: parameters of the interaction of antibody-antigen complexes with protein A. J. Immunol. **115**:1617–1624.
16. **Klemenz, R., and H. Diggelman.** 1978. The generation of the two envelope glycoproteins of Rous sarcoma virus from a common precursor polypeptide. Virology **85**:63–74.
17. **Laskey, R. A., and A. D. Mills.** 1975. Quantitative film detection of ^{3}H and ^{14}C in polyacrylamide gels by fluorography. Eur. J. Biochem. **56**:335–341.
18. **Levitt, R., S. Schlesinger, and S. Kornfeld.** 1977. Impaired intracellular migration and altered solubility of nonglycosylated glycoproteins of vesicular stomatitis virus and Sindbis virus. J. Biol. Chem. **252**:9018–9023.
19. **Lewandowski, L. J., R. E. Smith, E. P. Bolognesi, and M. S. Halpern.** 1975. Viral glycoprotein synthesis under conditions of glucosamine block in cells transformed by avian sarcoma virus. Virology **66**:347–355.
20. **Moelling, K., and M. Hayami.** 1977. Analysis of the precursors to the envelope glycoproteins of avian RNA tumor viruses in chicken and quail cells. J. Virol. **22**: 598–607.
21. **Nakamura, K., and R. W. Compans.** 1978. Effects of glucosamine, 2-deoxyglucose, and tunicamycin on glycosylation, sulfation, and assembly of influenza viral proteins. Virology **84**:303–319.
22. **Prochownik, W. V., S. Panem, and W. H. Kirsten.** 1975. Biological and physical modifications of a murine oncornavirus by 2-deoxy-D-glucose. J. Virol. **15**:1323–1331.
23. **Rifkin, D. B., and R. W. Compans.** 1971. Identification of the spike protein of Rous sarcoma virus. Virology **46**: 485–489.
24. **Scheele, C. M., and H. Hanafusa.** 1971. Proteins of helper-dependent RSV. Virology **45**:401–410.
25. **Schmidt, M. F. G., R. T. Schwarz, and H. Ludwig.** 1976. Fluorosugars inhibit biological properties of different enveloped viruses. J. Virol. **18**:819–823.
26. **Schmidt, M. F. G., R. T. Schwarz, and C. Scholtissek.** 1974. Nucleoside-diphosphate derivatives of 2-deoxy-D-glucose in animal cells. Eur. J. Biochem. **49**:237–247.
27. **Scholtissek, C.** 1971. Detection of an unstable RNA in chick fibroblasts after reduction of the UTP pool by glucosamine. Eur. J. Biochem. **24**:358–365.
28. **Schwarz, R. T., and H.-D. Klenk.** 1974. Inhibition of glycosylation of the influenza virus hemagglutinin. J. Virol. **14**:1023–1034.
29. **Schwarz, R. T., J. M. Rohrschneider, and M. F. G. Schmidt.** 1976. Suppression of glycoprotein formation of Semliki forest, influenza, and avian sarcoma virus by tunicamycin. J. Virol. **19**:782–791.
30. **Shealy, D. J., and R. R. Rueckert.** 1978. Proteins of Rous-associated virus 61, an avian retrovirus: common precursor for glycoproteins gp85 and gp35 and use of pactamycin to map translational order of proteins in the *gag*, *pol*, and *env* genes. J. Virol. **26**:380–388.
31. **Tozawa, H., H. Bauer, T. Graf, and H. Gelderblom.** 1970. Strain specific antigen of the avian leukosis sarcoma group. I. Isolation and immunological characterization. Virology **40**:530–539.
32. **Vogt, P. K.** 1969. Envelope classification of avian RNA tumor viruses. Comparative leukemia research. Bibl. Haematol. (Basel) **36**:153–167.
33. **Wang, L. H., P. H. Duesberg, S. Kawai, and H. Hanafusa.** 1976. Location of envelope-specific and sarcoma-specific oligonucleotides on RNA of Schmidt-Ruppin Rous sarcoma virus. Proc. Natl. Acad. Sci. U.S.A. **73**: 447–451.
34. **Weiss, R. A., W. S. Mason, and P. K. Vogt.** 1973. Genetic recombinants and heterozygotes derived from endogenous and exogenous avian RNA tumor viruses. Virology **52**:535–552.