JOURNAL OF VIROLOGY, Sept. 1976, p. 782-791
Vol. 19, No. 3

Printed in U.S.A.

Suppression of Glycoprotein Formation of Semliki Forest, Influenza, and Avian Sarcoma Virus by Tunicamycin

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Received for publication 21 May 1976

Tunicamycin, a new antibiotic, halts the formation of physical particles of Semliki forest and fowl plague virus, whereas avian oncornavirus particles which show a reduction in infectivity and do not contain detectable labeled glycoprotein are released in the presence of the drug. In Semliki forest virus-infected cells only the protein moieties of the glycoproteins could be labeled. In cells infected with fowl plague and avian sarcoma virus neither intact glycoproteins nor their protein moieties could be detected. By using a protease inhibitor (*N*-α-*p*-tosyl-L-lysin chloromethyl ketone, TLCK) it could be shown, however, that the carbohydrate-free hemagglutinin precursor of influenza virus is synthesized but is presumably degraded by intracellular proteases in the absence of TLCK as a consequence of the lack of carbohydrate.

To study the biological role of glycoproteins, inhibitors of glycosylation have become important tools. The most widely used inhibitors of virus glycoprotein formation are glucosamine and 2-deoxy-D-glucose (for review see reference 25). Two newly synthesized sugar analogues, 2-fluoro-2-deoxy-D-glucose and 2-fluoro-2-deoxy-D-mannose, also inhibit glycoprotein biosynthesis (23). As these chemicals are not exclusively interfering with glycosylation but are also analogues of sugars taking part in intermediary metabolism, side effects which vary depending on the concentration of the analogue and the cell type used must also be considered (M. F. G. Schmidt, Ph.D. thesis, Universitätsbibliothek der Justus Liebig-Universität, Giessen, 1975).

It was, therefore, desirable to work with more specific compounds which exclusively affect glycosylation without influencing other metabolic reactions. Tunicamycin seems to be the first compound fulfilling this demand. Tunicamycin is a glucosamine-containing antibiotic produced by *Streptomyces lysosuperificus* discovered and purified by Tamura and collaborators. It inhibits the multiplication of a variety of gram-positive bacteria, yeasts, and fungi and several different viruses (30, 32–35). As shown for *Bacillus subtilis* and chicken embryo fibroblasts, the biosynthesis of protein, RNA, and DNA is only slightly influenced, but the incorporation of sugar into the acid-insoluble macromolecular fractions is reduced (33). Kuo and Lampen (16) demonstrated that in the presence of tunicamycin the synthesis of the two external glycoproteins of yeast, invertase and acid phosphatase, is stopped. These reports prompted us to investigate biologically and biochemically the influence of tunicamycin on the formation of the glycoproteins of Semliki forest virus, fowl plague virus, and an avian oncornavirus.

MATERIALS AND METHODS

The Rostock strain of fowl plague virus (FPV) and the Osterrieth strain of Semliki forest virus (SFV) were grown in monolayers of either primary or secondary chicken embryo fibroblasts. Infected cells were maintained in minimal medium (26) containing 10 to 20 mM glucose.

For experiments with virus-transformed cells, the Prague strain of Rous sarcoma virus, subgroup C (PrC), was used to transform chicken embryo fibroblasts obtained from chicken helper factor-negative (10) 10-day-old C/BDE chicken embryos. The transformed cells were cultivated with Dulbecco's modification of Eagle medium (7) supplemented with 10% tryptose phosphate broth, 5% inactivated calf serum, and 1% dimethyl sulfoxide. Polybrene at 2 μg/ml was added at the time of infection to enhance virus adsorption (37).

Assays of viral activities. Plaque assays and hemagglutination titrations for FPV and SFV were carried out according to procedures described previously (39). The determination of neuraminidase activity of FPV was performed according to Drzeniek et al. (6). Focus formation for PrC was assayed according to Rubin (20).

Chemicals and isotopes. If not otherwise stated, chemicals were from E. Merck AG, Darmstadt, Germany. Reagents for polyacrylamide gels and *N*-α-*p*-tosyl-L-lysin chloromethyl ketone (TLCK) were from Serva, Heidelberg, Germany. Protein [U-^{14}C]hydrolysate (55 mCi/matom of carbon), D-[2-^{3}H]mannose (2 Ci/mmol), D-[1-^{14}C]glucosamine-hydrochlo-

ride (58 mCi/mmol), D-[6-^{3}H]glucosamine-hydrochloride (12 Ci/mmol), L-[4,5-^{3}H]leucine (57 Ci/mmol), L-[3,5-^{3}H]tyrosine (41 Ci/mmol), L-[2,3-^{3}H]-valine (16 Ci/mmol), and L-[1-^{3}H]fucose (3,3 Ci/mmol) were from Amersham Buchler, England.

C/BDE chicken embryos from fertile eggs supplied by Heisdorf and Nelson Farms (Redmond, Wash.) were obtained through William Mason and Robert Friis. Tryptose phosphate broth was from Difco Laboratories, Detroit, Mich. Polybrene was from EGA-Chemie KG, Steinheim/Albuch, West Germany.

Tunicamycin was a generous gift of G. Tamura, Laboratory of Microbiology, Department of Agricultural Chemistry, University of Tokyo, Japan, and monospecific antiserum against subgroup C gp85, the major envelope glycoprotein of the avian sarcoma virus, was a generous gift of D. Bolognesi, Duke University, Durham, N.C.

Labeling and analysis of viral proteins and particles. Confluent monolayers on plastic petri dishes (5-cm diameter) were infected with FPV or SFV and incubated in maintenance medium containing the desired concentration of tunicamycin. Two or four hours after infection the medium was supplemented by adding the radioactive isotopes. After 2 to 3 h (6 to 7 h postinfection), the medium was removed and the cells were washed three times with cold phosphate-buffered saline and processed for polyacrylamide gel electrophoresis as described (23); at 8 h postinfection the media were cleared of cell debris by centrifugation for 20 min at 4,000 rpm and pelleted by a 60-min 30,000 rpm centrifugation in the R40 rotor, and the pellets were resuspended in phosphate-buffered saline. After homogenization, the pellets were layered on top of a sucrose gradient in phosphate-buffered saline containing from 15 to 40% sucrose with a 1-ml cushion of 50% sucrose. The gradients were centrifuged in the SW41 swinging bucket rotor at 40,000 rpm for 2 h. After the run, the tubes were punctured and the gradients were fractionated (0.5-ml fractions).

For labeling PrC-infected cells, medium containing no tryptose phosphate broth and only 10% of the normal level of amino acids, and in which 10 mM fructose was substituted for glucose (27), was used (C. Scholtissek, personal communication). They were treated with 1 μg of tunicamycin per ml in labeling medium, and after 3 h ^{14}C-labeled protein hydrolysate and [2-^{3}H]mannose in a small volume were added to give final concentrations of 21 and 53 μCi/ml, respectively. After a further 5 h of incubation, the medium was harvested and virus was purified by sucrose gradient centrifugations. To analyze intracellular gp85, cell lysates were prepared and precipitation with specific antiserum was performed. The samples were prepared as described previously (19), and electrophoresis was performed according to the technique described by Laemmli (17). Determination of trichloroacetic acid-extractable and cell-bound radioactivity was done as described earlier (24).

RESULTS

Effect on infectivity and particle formation.

Rather low doses (<1 μg/ml) of tunicamycin in the culture medium of virus-infected cells inhibit the formation of infectious SFV and FPV, as can be detected by the plaque assay (Fig. 1). A rapid disappearance of functional hemagglutinin and neuraminidase in FPV-infected cells can also be seen (Fig. 1 inset). The media of PrC-infected cells showed a loss of infectivity after treatment with the antibiotic as quantitated by the focus formation test (Table 1). The host cells are not affected cytotoxically by the concentrations of tunicamycin used (34). To determine whether or not virus particles are formed in the presence of the antibiotic, infected cells were labeled from 2 to 8 h postinfection, and the medium was then analyzed. The results for FPV- and SFV-infected cells are shown in Fig. 2. In the media of nontreated cells, a peak of radioactivity migrates in the sucrose gradient to a position corresponding to

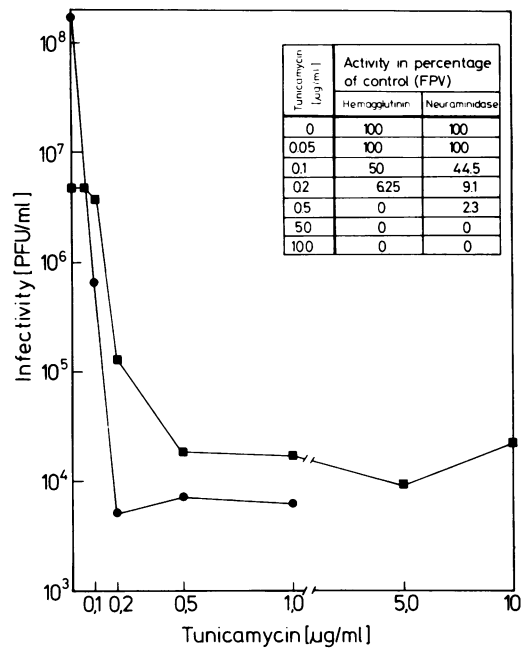


FIG. 1. *Dose-response of tunicamycin on the synthesis of infectious SFV and FPV and on the production of FPV-hemagglutinin and FPV-neuraminidase activity. Primary chicken embryo fibroblasts were infected with either SFV or FPV, and after a 1-h adsorption period tunicamycin was added at the concentrations indicated. Eight hours postinfection, the cells were scraped off and then frozen and thawed three times in the growth medium. Infectivity, hemagglutinin, and neuraminidase activities were determined as described (Materials and Methods), and the values are given in the figure as PFU per milliliter or percentage of control activity (inset), respectively. Symbols: ●——●, SFV titer; ■——■ FPV titer.*

TABLE 1. *Influence of tunicamycin on infectivity of avian sarcoma virus*[a]

Time (h)[b]	Titer (PFU/ml)		Infectivity (%)[c]
	−Tunicamycin	+Tunicamycin	
0–3	4.2×10^5	1.5×10^5	37
3–8	8.3×10^5	1.2×10^5	15
8–27	1.1×10^6	2.7×10^3[d]	

[a] Cells were washed three times with warm medium before addition of medium containing tunicamycin. At the time of the medium changes at 3 and 8 h, cells were washed twice with warm medium and the titers were determined on C/E chicken helper factor (−) cells.

[b] Interval after the addition of 1 μg of tunicamycin per ml.

[c] Percentage of infectivity in the medium of tunicamycin-treated cells as compared to the corresponding controls.

[d] Titer of sample from cells treated with tunicamycin at 0 to 8 h. No tunicamycin was present at 8 to 27 h, however.

infectious virus particles. The medium from tunicamycin-treated cells does not contain radioactivity in a similar position of the gradient. This result could be confirmed by analyzing the nonlabeled media of several roller flasks seeded with SFV- and FPV-infected cells. The media were processed as described (Materials and Methods), and the resulting pellets were centrifuged through sucrose gradients. The medium from nontreated FPV- and SFV-infected cultures contained UV-absorbing material which migrated together with infectivity. From the media of tunicamycin-treated cells no such material could be isolated (graphs not shown here).

The analysis of the media of treated and nontreated amino acid-labeled PrC-infected cells by sucrose density centrifugation shows that virus particles are being released at about 60% of the control level in the presence of the antibiotic (Fig. 3A, B). However, they show a slightly altered density and contain very little incorporated radioactive mannose (Fig. 3B) or glucosamine (not shown). These particles were analyzed by polyacrylamide gel electrophoresis for their protein composition as compared to normal particles. The viral proteins (p12, p15, p19, p23, p27) can be seen in their normal positions and almost normal amounts, whereas in the positions of gp85 and gp37 there is a decrease in the mannose incorporation to less than 1 and 3%, respectively, of that in the control virus (Fig. 3C, D). (The 23,000, dalton protein [p23], usually not detected by others, has also been observed in B 77 virus particles and is overproduced in a mutant of B 77, ts LA 334 [J. Rohrschneider et al., Virology, in press].) Since the glycoproteins do not label efficiently with amino acids and the background is high relative to the peak heights, these experiments cannot exclude the possibility that the newly synthesized protein portions of gp85 and gp37, which would presumably migrate with increased mobility, are present at approximately 40% or less of the normal amounts.

Effect on intracellular protein synthesis. To answer the question of whether the nonglycosylated forms of gp85 were synthesized but not incorporated into virions, labeled cell lysates were mixed with anti-gp85 serum and the resulting precipitates were analyzed by gel electrophoresis. In the case of tunicamycin-

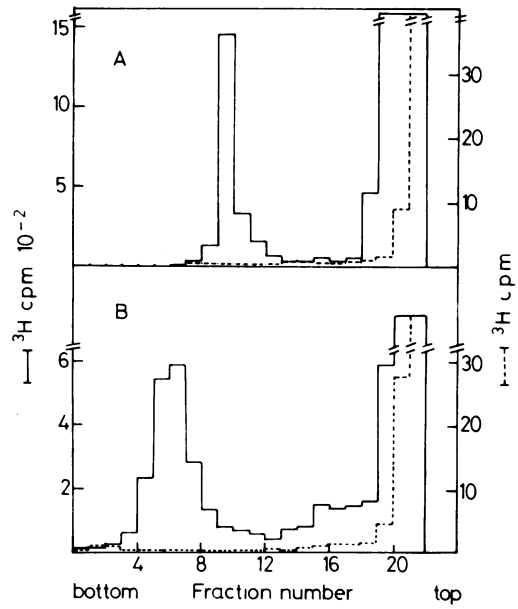


FIG. 2. *Analysis of physical particles in the media of (A) SFV- and (B) FPV-infected cells. Secondary chicken embryo fibroblasts were labeled from 2 to 8 h postinfection with a mixture of tritiated leucine, valine, and tyrosine (total of 50 μCi) in Earle medium in the presence of 1 μg of tunicamycin per ml or without the inhibitor. The media were cleared of cell debris by low-speed centrifugation, the supernatants were pelleted, and the pellets were resuspended in phosphate-buffered saline. After homogenization they were centrifuged to equilibrium in a sucrose gradient from 15 to 40% with a 1-ml cushion of 50% sucrose. The gradients were fractionated, and portions were counted for radioactivity. The maximum of infectivity in the controls was found in fractions 10 (A) and 7 (B), respectively. Symbols: ——, media from nontreated cells; -----, media from tunicamycin-treated cells.*

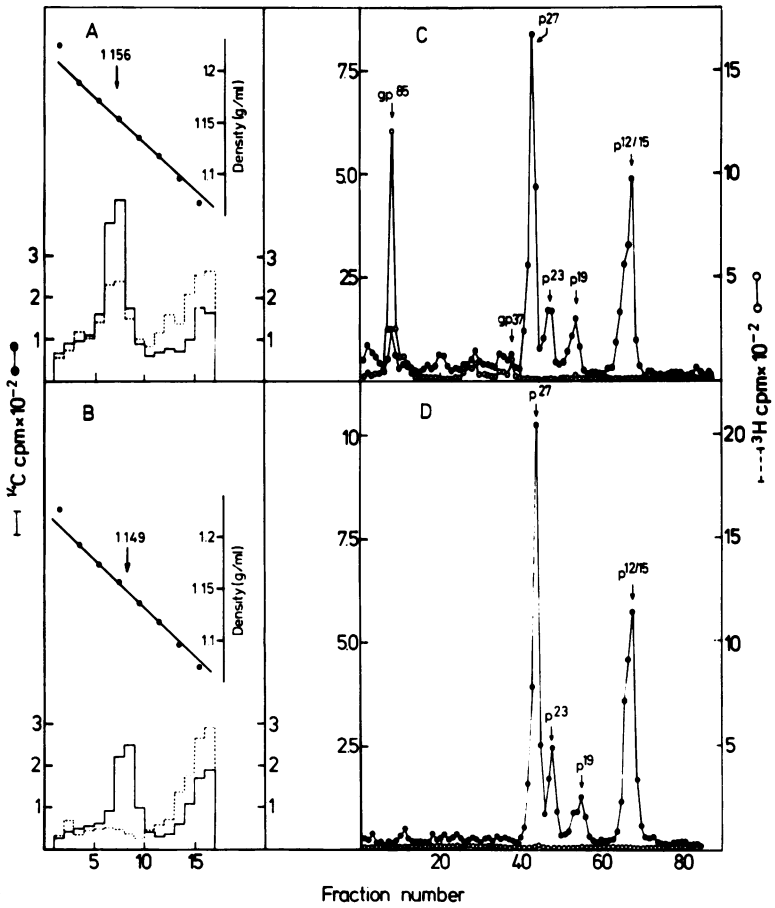
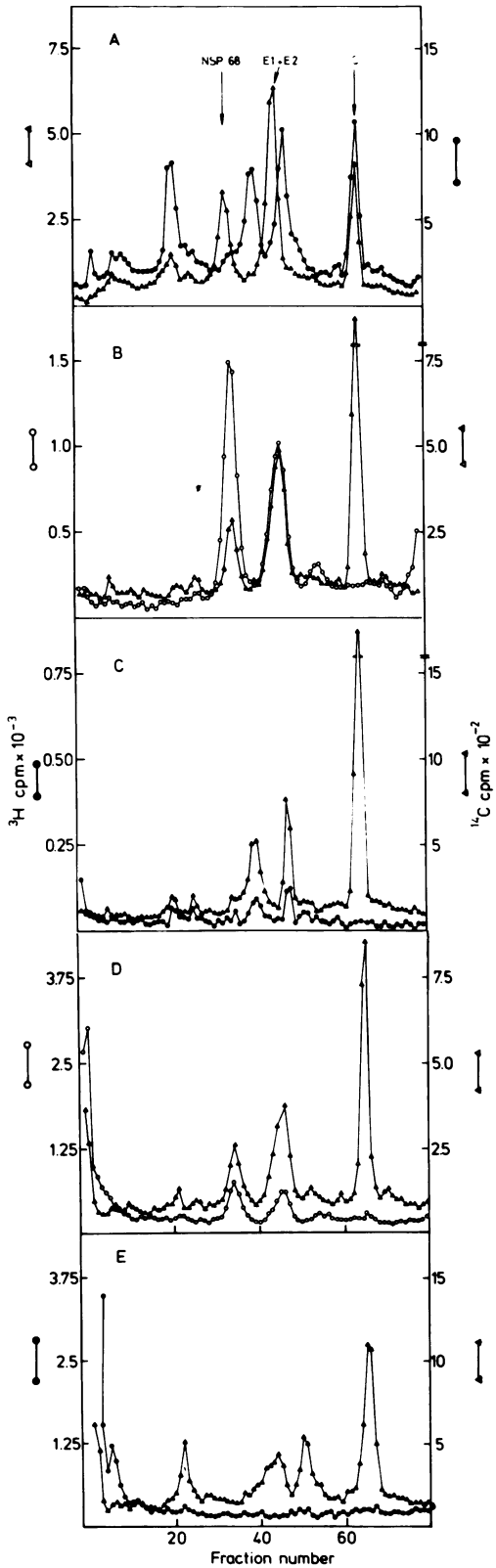


FIG. 3. *Analysis of physical particles from the media of PrC-infected cells. PrC-transformed cells were treated with tunicamycin (1 μg/ml) or left untreated for 3 h. Radioactive isotopes were then added, and after an additional 5 h of incubation the media were harvested and cleared of cell debris by centrifugation at 10,000 rpm for 10 min, and the virus was pelleted by a 1-h centrifugation at 35,000 rpm in the SW50.1 rotor. The pellets were resuspended in TNE (10 mM Tris-100 mM NaCl-1 mM EDTA) at pH 7.4 and centrifuged to equilibrium in a 20 to 50% sucrose gradient for 17 h. The gradients were fractionated, and portions were counted for radioactivity. The density of alternate fractions was determined by reading the refractive index (A, nontreated; B, tunicamycin-treated). Peak fractions were pooled, centrifuged at 35,000 rpm for 1 h, and taken up in TNE. Portions were precipitated with a 10-fold excess of cold acetone and centrifuged at 2,500 rpm for 10 min, and the precipitated material was taken up in Laemmli sample buffer. The samples were subjected to electrophoresis on 9-cm gels (13% Laemmli separating gels with 1-cm 3% stacking gels). Electrophoresis was at 1 mA/gel through the stacking gel and 3 mA/gel through the separating gel (C, nontreated; D, tunicamycin-treated). Symbols: -----, [^{3}H]mannose (A, B); ——, ^{14}C-labeled amino acid (A, B); ○, [^{3}H]mannose (C, D); ●, ^{14}C-labeled amino acid (C, D).*

treated cells no gp85 could be detected, in contrast to the lysates from control cells, which contained high amounts of gp85, given the same limitations as discussed above (results not shown here).

Cells infected with SFV or FPV were analyzed to determine whether the lack of virus particle formation in these systems in the presence of tunicamycin is due to a failure of biosynthesis of viral proteins. The infected cells were labeled and analyzed by polyacrylamide gel electrophoresis. It can be seen that 1 μg of tunicamycin per ml in the culture medium leads to a shift of the viral glycoproteins of SFV (NSP68 and E_1 plus E_2) to positions which are compatible with lower molecular weights as evaluated by gel electrophoresis when compared to control lysates (Fig. 4A, closed circles). This finding is similar to the results described for 2-deoxy-D-glucose and 2-deoxy-2-fluoro



sugars (12, 23). The core protein which is not glycosylated is found in the same position in treated as in nontreated cells. In addition there is an accumulation of a protein with a molecular weight between 85,000 and 95,000 (fractions 22 to 24 in Fig. 4A) in the treated cells. To examine the extent of glycosylation of the SFV glycoproteins, infected cells were labeled in the presence or absence of the inhibitor with tritiated mannose or glucosamine or with ^{14}C-labeled protein hydrolysate. Portions of the cell lysates were mixed and subjected to co-electrophoresis. One can see from Fig. 4C and E that the protein label in the glycoproteins is not reduced. The incorporation of carbohydrate label into the glycoproteins, however, is almost completely inhibited.

In FPV-infected cells, the hemagglutinin glycoproteins can not be detected in the presence of tunicamycin either in the glycosylated or in the nonglycosylated form (Fig. 5A, closed circles), whereas other viral proteins such as NP and M are still being synthesized. In accordance with this finding, there is no radioactivity found in the gel regions of the FPV-glycoproteins when ^{14}C-labeled sugars are used for labeling of the cells in the presence of tunicamycin (Fig. 5C and E).

The question arises as to whether not only the glycosylation but also the synthesis of the protein part of the FPV glycoproteins is stopped in the presence of tunicamycin. To test whether

FIG. 4. *Sodium dodecyl sulfate-polyacrylamide gel electrophoresis pattern of proteins and glycoproteins of chicken cells infected with SFV in the presence of or absence of tunicamycin. Secondary chicken embryo fibroblasts were labeled at 4 h postinfection for 2 (panel A) or 3 h (panels B–E) with 25 μCi of a mixture of tritiated amino acids per ml, 1 μCi of ^{14}C-labeled protein hydrolysate per ml, and 25 μCi of [2-^{3}H]mannose or [6-^{3}H]glucosamine per ml in a total volume of 2 ml. Tunicamycin was added at 1 h postinfection. After the labeling period the cells were processed for electrophoresis as described in Materials and Methods. Portions of the lysates were mixed and applied to 8.75% polyacrylamide gels for co-electrophoresis. (A) SFV-specific proteins labeled with ^{14}C-labeled protein hydrolysate in the presence of 1 μg of tunicamycin per ml (●) or with tritiated amino acids in the absence of the drug (△). (B) SFV-specific proteins from nontreated cells labeled with ^{14}C-labeled protein hydrolysate (△) or with [2-^{3}H]mannose (○). (C) Labeling was as given in panel B, but cells were grown in the presence of 0.1 μg of tunicamycin per ml (closed symbols). (D) Virus-specific proteins from nontreated cells labeled with ^{14}C-labeled protein hydrolysate (△) or with [6-^{3}H]glucosamine (○). (E) Labeling as given for panel D but in the presence of 1 μg of tunicamycin (closed symbols) per ml. The arrows mark the positions of the three virus-specific glycoproteins NSP 68 (p-62), E_1, and E_2 and the position of the core protein (1).*

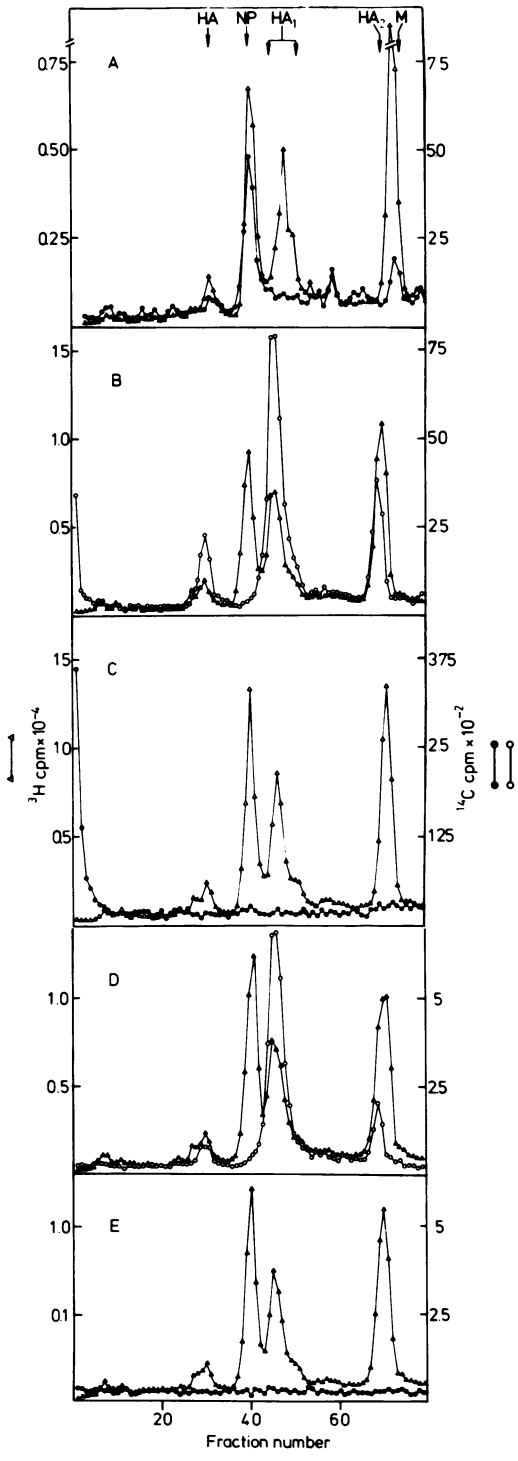


FIG. 5. *Sodium dodecyl sulfate-polyacrylamide gel electrophoresis pattern of proteins and glycoproteins of chicken cells infected with FPV in the presence of or without tunicamycin. Secondary chicken embryo fibroblasts were labeled at 4 h postinfection for 3 h with 1 μCi of ^{14}C-labeled protein hydrolysate, 25 μCi of tritiated amino acids per ml, or 5 μCi of ^{14}C-labeled sugars in a total of 2 ml of Earle medium with (closed symbols) or without 1 μg of tunicamycin per ml (open symbols). Portions of the ^{14}C-labeled cell lysates were mixed with a ^{3}H-labeled control lysate (△) for co-electrophoresis. Addition of tunicamycin was at 1 h postinfection. (A) Virus-specific proteins labeled with ^{14}C-labeled protein hydrolysate in the presence of tunicamycin (●). (B) Virus-specific proteins labeled with ^{14}C-labeled glucosamine (○). (C) Virus-specific proteins were labeled as given for (B) but in the presence of tunicamycin (●). (D) Virus-specific proteins were labeled with [^{14}C]fucose (○). (E) Labeling was as given for (D) but in the presence of tunicamycin (●).*

the protein moieties of the FPV glycoproteins are synthesized when tunicamycin is present in the medium, FPV-infected chicken cells were treated with the protease inhibitor TLCK (28). This treatment leads to a block of cleavage of the hemagglutinin in the FPV-infected control cells which therefore accumulates (Fig. 6A). In the presence of TLCK in tunicamycin-treated FPV-infected cells, the nonglycosylated form of hemagglutinin (14, 15, 28) can be detected (Fig. 6B). This finding supports the view that the protein moiety of the hemagglutinin is synthesized in the presence of tunicamycin but is extremely labile to endogenous proteases and therefore not detectable in the absence of the protease inhibitor.

Effect on uptake and incorporation of sugars. The results of experiments are shown in which the uptake and incorporation of sugar label into SFV-infected cells were monitored (Fig. 7). Although the uptake of radioactive mannose in tunicamycin-treated cells was only decreased by approximately 40% (Fig. 7A), incorporation of this sugar into trichloroacetic acid-precipitable material was inhibited by more than 95% (Fig. 7C). Using radioactive glucosamine as sugar label no significant alteration of the uptake could be detected in the presence of the inhibitor (Fig. 7B), whereas in contrast to the results with mannose the incorporation of glucosamine was only inhibited by less than 25% in many experiments (Fig. 7D). This finding shows that general incorporation of glucosamine at least in SFV-infected cells is not inhibited, whereas the glycosylation of SFV-specific glycoproteins with glucosamine seems to be almost completely suppressed by tunicamycin (Fig. 4D, E).

DISCUSSION

The effect of the antibiotic tunicamycin on cells infected with different glycoprotein-containing viruses is essentially the same for the

viruses tested as far as the reduction of infectivity and disturbance of glycoprotein synthesis is concerned. In the case of SFV- and FPV-infected cells, tunicamycin inhibits the formation of virus particles. However, it can be shown by gel electrophoresis that in lysates of tunicamycin-treated and labeled cells all viral proteins are present after SFV infection. The glycoproteins NSP 68 and E_1 plus E_2 show an increased electrophoretic mobility when synthesized in the presence of tunicamycin, which is compatible with a decreased carbohydrate content (13). The core protein which is not a glycoprotein does not show such an alteration. By gel electrophoresis of lysates of FPV-infected and tunicamycin-treated cells, one can see the FPV proteins (NP and M) in their appropriate positions in the gel, whereas the essentially carbohydrate-free forms of the hemagglutinin proteins (14, 15, 28) are not visible, except in the presence of the protease inhibitor TLCK, where nonglycosylated hemagglutinin accumulates. These findings support the view that the nonglycosylated forms of the different virus glycoproteins show different stabilities within the same host cell (chicken embryo fibroblasts). The protein moieties of SFV glycoproteins are stable, whereas those of FPV are degraded completely. The somewhat higher stability of the FPV-glycoproteins in the 2-deoxy-D-glucose-mediated inhibition of glycosylation (28) might be due to the fact that in the presence of the sugar analogue, trace amounts of carbohydrates are still being attached to the protein part of the molecules. This could prevent the complete breakdown of these molecules,

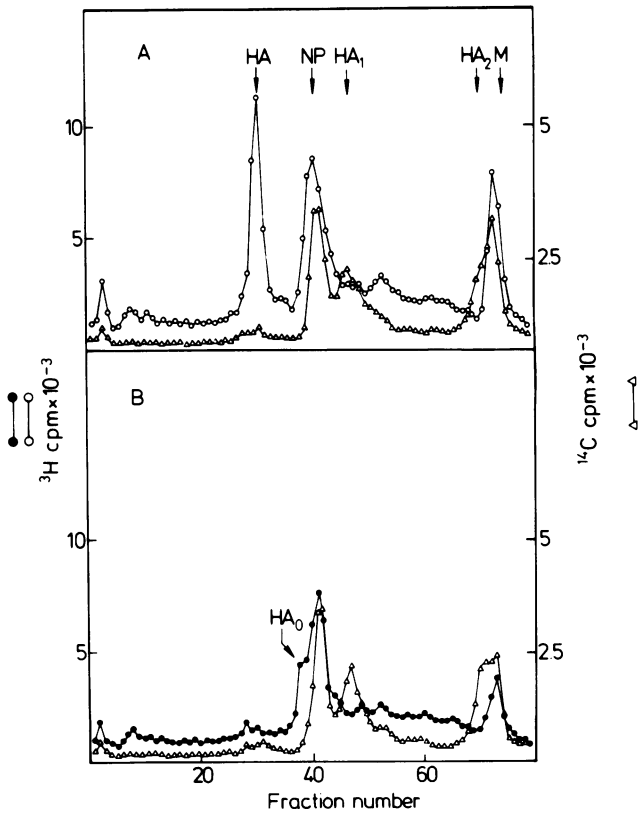


FIG. 6. *Accumulation of nonglycosylated hemagglutinin in FPV-infected chicken cells in the presence of tunicamycin and TLCK. Secondary chicken embryo fibroblasts were infected with FPV and after the adsorption period (1 h postinfection) treated with 1 μg of tunicamycin per ml (closed symbols) or grown in normal Earle medium (open symbols). TLCK was applied at 3 h 45 min postinfection at a concentration of 0.5 mM, and 15 min later 25 μCi of tritiated amino acids per ml was added. To the control plate, 2 μCi of ^{14}C-labeled protein hydrolysate was added to a total volume of 2 ml of Earle medium. After labeling for 3 h the cells were processed for polyacrylamide gel electrophoresis in 8.75% gels using 3 ml of 2% stacking gel. Lystates of ^{3}H-labeled amino acid-labeled TLCK-treated cells (panel A, circles) or of ^{3}H-labeled amino acid-labeled TLCK plus tunicamycin cells (panel B, closed circles) were mixed with lysates of ^{14}C-labeled protein hydrolysate-labeled control cells (panels A and B, triangles) for co-electrophoresis.*

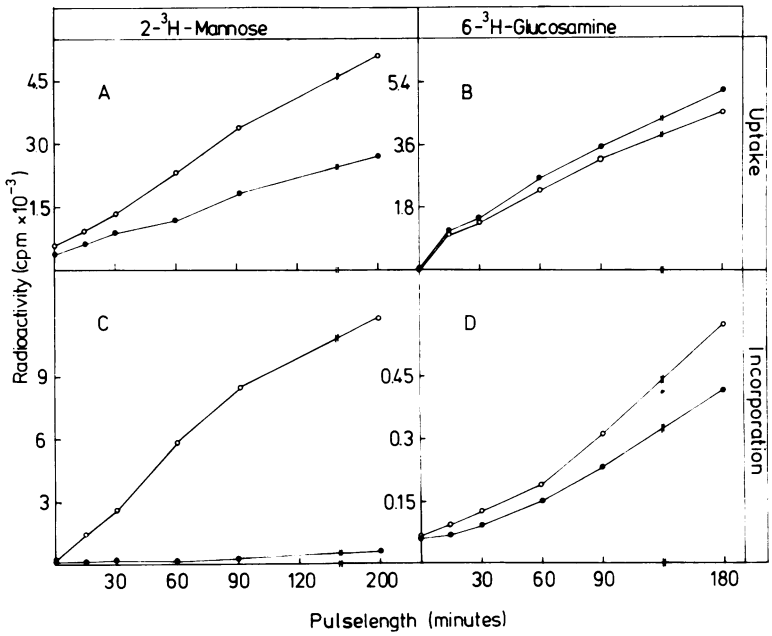


FIG. 7. *Influence of tunicamycin on the uptake and incorporation of [2-³H]mannose and [6-³H]glucosamine into SFV-infected cells. Primary chicken embryo fibroblasts in 3.5-cm dishes were labeled at 4 h postinfection either with 5 μCi of [2-³H]mannose (A, C) or with 2.5 μCi of [6-³H]glucosamine (B, D) in a total volume of 1 ml of Earle medium containing 1 μg of tunicamycin per ml (closed symbols) or no inhibitor (open symbols). At the indicated times the cultures were processed according to Scholtissek (24), and radioactivity was determined in the trichloroacetic acid-soluble and trichloroacetic acid-precipitable fractions. Each value represents the mean from two cultures analyzed.*

whereas in the tunicamycin-mediated inhibition the influenza glycoproteins might be completely free of sugar and therefore highly susceptible to proteolytic attack.

In the presence of the antibiotic, avian sarcoma virus-infected cells release viral particles which do not contain detectable levels of newly synthesized, radioactively labeled glycoproteins (gp85 or gp37) either in the glycosylated or in the nonglycosylated form as analyzed by gel electrophoresis. If one analyzes in addition the tunicamycin-treated PrC-infected and labeled cells, it is found that as in the influenza-infected cells, probably also due to proteolytic lability, neither glycosylated nor nonglycosylated glycoproteins can be detected (one limitation of this statement being that the nonglycosylated gp85 might not be precipitated with the antiserum prepared against fully glycosylated gp85).

Considering the dramatic decrease in [³H]mannose incorporated into gp85 and gp37 in the presence of tunicamycin, we were surprised that the infectivity has decreased to only 15% of the control values for the comparable time period (3 to 8 h after administration of the drug). This result has led us to the supposition, which is now being tested, that either complete virus particles which adhere to the cell surface despite extensive washing or glycoproteins which are embedded in the membrane, having been synthesized prior to the radioactive pulse, are responsible for the relatively high level of infectivity in particles released between 3 and 8 h after the addition of tunicamycin. We therefore cannot at this time decide on the role of glycoproteins in the formation of physical particles of Rous sarcoma virus. It is known, however, that a mutant of Rous sarcoma virus, BH-RSV, forms noninfectious particles with little or no gp85 or gp37 but which do contain a new glycoprotein of unknown origin with a molecular weight of 43,000 (9, 21). We are presently performing additional experiments to determine whether similar particles may be released after extended exposure of PrC-infected cells to tunicamycin.

Especially in SFV- or FPV-infected chicken cells, and probably also in PrC-infected cells, tunicamycin-mediated inhibition of glycosylation is correlated with a considerable loss of infectivity. This observation is not incompatible with the results published recently by Schlesinger et al. (22), who found infectious

Sindbis virus particles released from cell mutants which lack a specific glucosaminyl transferase. The virus particles produced in these mutant cells lacked part of their sugar content and contained envelope glycoproteins of lower molecular weight when compared to Sindbis virus grown in the wild-type cells. The authors conclude that although glycosylation does not need to occur to completion, at least part of the sugars, the core sugars, seem to be critical for the biosynthesis of infectious particles. In the presence of tunicamycin, however, not even the core sugars seem to be incorporated into the viral glycoproteins.

A detailed explanation for the mechanism of tunicamycin in virus-infected cells cannot yet be given. Two recent reports, however, could demonstrate that in vitro the formation of polyisoprenol sugars can be prevented by tunicamycin (31, 36).

As the uptake and the activation of the sugars is not inhibited in our virus-cell system (unpublished data), we suppose that polyisoprenyl sugars may be involved in the biosynthesis of virus glycoproteins at least for the formation of the core sugars (2, 3, 11, 18, 38). The sugars located at the periphery are most likely added by stepwise addition which is in accordance with results previously published (4, 15, 22, 29). As there is only a slight inhibition of glucosamine incorporation into SFV-infected chicken embryo cells as a whole in the presence of tunicamycin, whereas virus glycoproteins are not glycosylated with this sugar under the same conditions, it has to be assumed that glucosamine is utilized for the biosynthesis of other cellular macromolecules such as mucopolysaccharides. These alternative pathways of glucosamine are not affected by the antibiotic.

With the carbohydrate-free protein moieties of the glycoproteins, it should be possible to study the contribution of the protein and the sugar part to the immunological and other properties of glycoproteins. Furthermore, these molecules appear well suited to serve as specific acceptors for in vitro studies of glycosylation.

ACKNOWLEDGMENTS

We thank Irene Weibel, Juliana Winkler, Sigrun Bingel, and Michaela Orlich for their excellent technical assistance. We thank R. Rott, C. Scholtissek, H.-D. Klenk, R. R. Friis, and H. Bauer for continuous support, stimulating discussions, and critical reading of the manuscript.

We are greatly indebted to G. Tamura, Laboratory of Microbiology, Department of Agricultural Chemistry, University of Tokyo, Japan, for a generous gift of tunicamycin.

We also thank D. Bolognesi, Duke University, Durham, North Carolina, for monospecific gp85 antiserum.

This work was supported by the Sonderforschungsbereich 47 (Virologie).

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