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Effect of Tunicamycin on Herpes Simplex Virus Glycoproteins and Infectious Virus Production

LEWIS I. PIZER,[1]†* GARY H. COHEN,[2] AND ROSELYN J. EISENBERG[2]‡

Department of Microbiology, School of Medicine,[1] and Department of Microbiology, School of Dental Medicine and the Center for Oral Health Research,[2] University of Pennsylvania, Philadelphia, Pennsylvania 19104

The antibiotic tunicamycin, which blocks the synthesis of glycoproteins, inhibited the production of infectious herpes simplex virus. In the presence of this drug, [^{14}C]glucosamine and [^{3}H]mannose incorporation was reduced in infected cells, whereas total protein synthesis was not affected. Gel electrophoresis of [2-^{3}H]mannose-labeled polypeptides failed to detect glycoprotein D or any of the other herpes simplex virus glycoproteins. By use of specific antisera we demonstrated that in the presence of tunicamycin the normal precursors to viral glycoproteins failed to appear. Instead, lower-molecular-weight polypeptides were found which were antigenically and structurally related to the glycosylated proteins. Evidence is presented to show that blocking the addition of carbohydrate to glycoprotein precursors with tunicamycin results in the disappearance of molecules, possibly due to degradation of the unglycosylated polypeptides. We infer that the added carbohydrate either stabilizes the envelope proteins or provides the proper structure for correct processing of the molecules needed for infectivity.

The contribution of the carbohydrate portion of viral glycoproteins to infectivity, antigenicity, and virus-mediated cell fusion constitutes an interesting area of research which has far-reaching biological implications. The antibiotic tunicamycin (TM) (38), which blocks the synthesis of the *N*-acetylglucosamine-lipid intermediates required for glycoprotein synthesis (36, 39, 41), provides a useful and specific tool for investigating the role of glycoproteins in these viral processes. Several laboratories have made use of TM to investigate the synthesis of enveloped RNA viruses. They found that the reduction of glucosamine incorporation caused by TM was accompanied by a decrease in infectious virus production, whereas the synthesis of viral macromolecules in general was not drastically lowered in antibiotic-treated cells (21, 32, 40). Recently, TM has been used to study the maturation of viral glycoproteins. Investigations with vesicular stomatitis virus (12, 28, 44), Semliki Forest virus (11), and influenza virus (23), as well as murine (44) and avian tumor viruses (8), have shown the presence of nonglycosylated forms of viral glycoproteins; in some instances the nonglycosylated proteins were inserted into membrane structures (11, 12, 23). From the evidence available, it appears that TM has a marked effect on glycoprotein synthesis in enveloped RNA virus-infected cells. The results are consistent with the biochemical data obtained with other systems, namely that TM inhibits the attachment of *N*-acetylglucosamine to dolichol phosphate and hence prevents addition of oligosaccharide chains to proteins (36, 43).

Herpes simplex virus (HSV), an enveloped DNA virus, contains glycoproteins as structural components. Investigators have characterized these glycoproteins in purified virions (14, 18, 26, 35), studied their biosynthesis in infected cells (15, 34) and inferred a variety of biological functions from mutant or antimetabolite studies (7, 10, 17, 20, 22, 30, 31). There is general agreement that there are four HSV type 1 glycoproteins, which fall into two molecular weight classes, 50,000 to 60,000 and 110,000 to 130,000. Spear (34) designated the three glycoproteins in the 130,000-molecular-weight region as glycoproteins gA, gB, and gC, and designated the glycoprotein in the 60,000-molecular-weight region as gD. This terminology was accepted provisionally at the 1979 Herpes Virus Workshop in Cold Spring Harbor. Recently, the genetic loci specifying the HSV glycoproteins have been established (29), and involvement of individual glycoprotein species in cell-to-cell interactions has been described (22, 29).

Previously (3, 4), we reported the isolation of

† Present address: Department of Microbiology and Immunology, University of Colorado Medical Center, Denver, CO 80262.

‡ Present address: Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA 19104.

a HSV-specific fraction (designated CP-1) from HSV type 1-infected cells having the properties of a glycoprotein. Antiserum to CP-1 was able to neutralize the infectivity of both HSV types 1 and 2. Recent studies showed that CP-1 activity is associated with a 52,000-dalton (52K) glycoprotein (gp52) synthesized in the infected cell and with a 59K glycoprotein (gp59) in the mature virion (3). The precursor form of CP-1, gp52, has been designated as pgD(52), and the gp59 product has been designated gD. Subsequently we demonstrated that pgD(52) and gD share methionine and arginine tryptic peptides and that pgD(52) contains an 1,800-dalton oligomannosyl core (9). The oligosaccharide core on pgD(52) is processed by glycosylation and sialylation to a larger, more heterogeneous oligosaccharide on the mature glycoprotein gD (9).

These results are in agreement with the proposal of Spear (34) and of Honess and Roizman (15) that HSV glycoproteins exist in both precursor and product forms in the infected cell. Furthermore, the biochemical changes that underly the conversion of pgD to gD are similar to the maturation steps described for other complex viral glycoproteins, such as the G protein of vesicular stomatitis virus (16, 27, 37).

In this report we present evidence that TM inhibits glycosylation of HSV glycoproteins and the production of infectious virus. By use of specific antisera we have demonstrated that in the presence of TM the normal precursors to viral glycoproteins fail to appear. Instead, lower-molecular-weight polypeptides were found which are antigenically and structurally related to the glycosylated proteins. These polypeptides, which lack carbohydrates, appear to be somewhat unstable. (A preliminary report of this work was presented at the ICN-UCLA Symposia on Biological Recognition and Assembly, 4–19 March 1979, Keystone, Colo.)

MATERIALS AND METHODS

Cells and virus. Baby hamster kidney cells (BHK/C13) were obtained from the Virology Institute, Glasgow, Scotland. They were grown in Eagle minimal medium supplemented with 10% fetal calf serum, penicillin, and streptomycin. Periodic tests for mycoplasma were negative. Experiments were carried out with rapidly growing cells at low to medium cell densities. HSV type 1 strain HF, which causes syncytium formation, was used throughout, and 1 mM arginine was added to cultures before infection with HSV. The procedures for viral growth, titration, and storage have been described (5).

Labeling procedures. To follow the rate of protein and glycoprotein synthesis, the incorporation of L-[^{35}S]methionine (600 Ci/mmol) or D-[1-^{14}C]glucosamine (57 Ci/mmol) (New England Nuclear Corp.) into acid-precipitable material was measured. Sterile glass cover slips (2.5 cm^2) were placed in 3-cm petri dishes, and suspensions of BHK cells were allowed to attach to the glass surface. Three days later, the medium was replaced, and the experiment was performed the following day. After the number of cells present on the cover slips was determined, the medium was removed, and the appropriate amount of virus in 0.1 ml was added to give a virus multiplicity of 10 to 20 PFU/cell. Adsorption proceeded for 90 min before the viral suspension was removed and replaced with 0.9 ml of fresh medium containing radioactive compounds and 1/10 the normal methionine. TM, when added, was included in the radioactive medium at a final concentration of 2 μg/ml. At the times indicated, duplicate or triplicate cover slips were removed from the radioactive medium and washed with cold phosphate-buffered saline, 6% trichloroacetic acid, and ethanol. After drying at 60°C, the dried cover slips with cell residue attached were counted in a scintillation counter.

To obtain cytoplasmic extracts for antibody precipitation, cells were labeled with L-[^{35}S]methionine (600 Ci/mmol), L-[2,3-^{3}H]arginine (21.7 Ci/mmol) or D-[2-^{3}H(N)]mannose (18.4 Ci/mmol) and disrupted by the procedure of Vogt et al. (42). A single 6-cm or 10-cm plate of cells was used for each sample. For long-term labeling (4 to 6 h), the medium contained 1% serum and 1/10 the normal amount of methionine. For pulse labeling (15 or 30 min), the conditions described by Vogt et al. (42) were used, and to each plate was added 100 μCi of [^{35}S]methionine, 500 μCi of [^{3}H]arginine, or 1 mCi of [^{3}H]mannose. All radioactive compounds were purchased from New England Nuclear Corp. (Boston, Mass.). The incorporation of these compounds into an acid-precipitable form was measured by placing a sample onto a paper disk, washing with trichloroacetic acid followed by ethanol, and counting with a toluene-based scintillation solution in a scintillation counter. Slices of dried gels were counted directly under the same conditions. Standardized gels demonstrated that under the conditions used the radioactivity in the gel slices accurately reflected the amount of labeled protein electrophoresed. The protease inhibitors L-1-tosylamide 2-phenethyl chloromethyl ketone and *N*-α-*p*-tosyl-L-lysine chloromethyl ketone were added to the cytoplasmic extract at the time of breakage of the cells, each at a final concentration of 1 mM, and were included in the buffers used to wash the immune precipitates.

Antibody precipitation. Antiserum against the envelope fraction from purified virions (anti-ENV-1 serum) was prepared in rabbits. A description of this antiserum and its use in precipitation of viral proteins has recently been published (3, 9). The previous procedure was modified in that a preparation of *Staphylococcus aureus* Cowan strain I was used to collect antigen-antibody complexes (19, 33). Antiserum and *S. aureus* were added in excess to ensure complete precipitation of antigens. After thorough washing with lysis buffer supplemented with 0.1% sodium dodecyl sulfate, antibody-antigen precipitates were dissociated by heating (80°C) in 3% sodium dodecyl sulfate buffer and subjected to electrophoresis on 12.5% acrylamide gels crosslinked with *N,N'*-diallyltartardiamide (14). After electrophoresis the gels were fixed, dried, and

exposed to Kodak XRP-5 X-ray film. When tritiated compounds were used, the gel was prepared for fluorography by the method of Bonner and Laskey (2). The molecular weights of the viral polypeptides were calculated with the aid of a set of molecular weight markers run on the same gel (3, 9). We believe that for the HF strain of HSV type 1 the 120K glycoprotein band is gA/gB, the 130K glycoprotein is gC, and the 59K glycoprotein is gD. There may be some uncertainty about the 130K glycoprotein, but for the purposes of this paper we will designate it as gC.

Tryptic peptide analysis. Proteins were eluted from gels and digested with trypsin, and the peptides were chromatographed on Chromobeads P using the procedures described by Vogt et al. (42), modified according to Eisenberg et al. (9). The 154K viral polypeptide obtained from purified capsids labeled with [^{35}S]methionine was added as an internal marker for chromatography. The peptides generated from the 154K polypeptide provide reference points on each chromatogram. The arrows on the figures indicate where elution with pH 4.9 buffer was started.

RESULTS

Virus production. The replication of HSV was markedly inhibited by 2 μg of TM per ml added 90 min after infection (Fig. 1). In this experiment, the control culture yielded about 50 PFU/cell, whereas the cultures treated with TM failed to produce appreciably more virus than the background arising from cell-associated input virus (0.5 to 1.0 PFU/cell). A similar result was obtained when the antibiotic was added either at the same time as the virus or 3 h before infection. Adsorption of virus was not altered in the pretreated cells, nor could the effect of TM be reversed by extensive washing of these cells with medium just before the time of adding virus.

Effect of TM on the incorporation of precursors into protein and glycoprotein. The time course of [^{14}C]glucosamine and [^{35}S]methionine incorporation into infected and uninfected cells is shown in Fig. 2. It is evident that TM rapidly reduced glucosamine incorporation to the same extent (75 to 85%) in both the infected and uninfected cells. Altering the TM concentration from 1 to 10 μg/ml (results not shown) did not change the degree of inhibition nor suggest any reduction in antibiotic sensitivity after infection. These results indicate that the synthesis of viral glycoproteins involves the same or similar dolichol-mediated sugar transfer reactions present in uninfected cells (36, 43).

An unlikely alternative, that TM is affecting overall viral protein synthesis, is ruled out by the methionine incorporation data, which show that protein synthesis occurs in TM-treated infected cells to the same extent as in nontreated infected cells (Fig. 2B). However, the rate of protein synthesis in uninfected cells was inhibited more than 50% by TM.

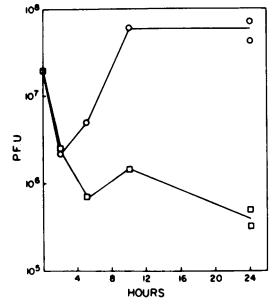

FIG. 1. *Growth curve. Growing cells in 30-mm plates (5 × 10^5 cells per plate) were infected at an input multiplicity of 60 PFU/cell. After 90 min of adsorption, the virus was removed, and the plates were washed thoroughly with warm medium and overlaid with 2 ml of medium with or without 2 μg of TM per ml. From the titer of unadsorbed virus an effective multiplicity of 20 PFU/cell was calculated. Plates were harvested at the indicated times, and the virus produced was determined by duplicate titrations.* (○) *Control;* (□) *treated.*

Electrophoretic patterns of radioactive cytoplasmic polypeptides synthesized in the presence and absence of TM were similar except that bands corresponding to the viral glycoproteins at 59K (gD) and 130K region were absent or greatly reduced, and new bands at about 50K and in the 80K to 110K region appeared (Fig. 3, lanes A and B).

Immunoprecipitation of HSV glycoprotein. Using antiserum against the envelope from purified virions (anti-ENV-1) improved our ability to distinguish glycoproteins in cell extracts and to follow the effect of TM on their synthesis. Initially the direct precipitation procedure used previously to study HSV glycoproteins was employed (3, 9). The results of pulse-chase experiments with [^{35}S]methionine indicated that polypeptides at 50K and 85K were synthesized in the presence of TM that were antigenically related to viral glycoproteins (data not shown). To improve the sensitivity of the method, the pulse-chase labeling experiments were repeated with anti-ENV-1 serum and washed *S. aureus* to enhance precipitation of antibody-antigen complexes.

As shown in Fig. 3, this procedure detected radioactive bands in immune precipitates from cells labeled for 30 min with [^{3}H]arginine. In untreated cell extracts, heavily labeled bands with molecular weights of about 52K, 110K, and 120K were observed (Fig. 3, lane C). Bands containing less radioactivity, corresponding to polypeptides of about 65K, 85K, and 130K, were

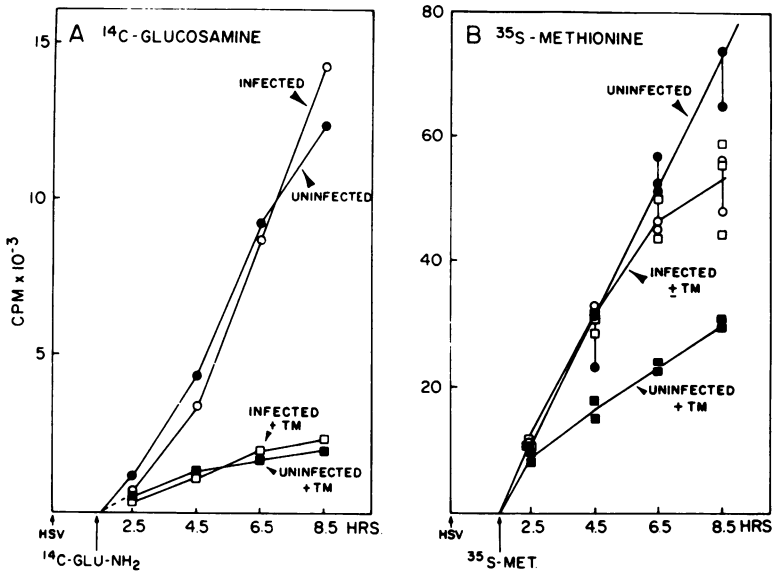

FIG. 2. *Glucosamine and methionine incorporation into macromolecules. Cells growing on cover slips were infected or mock-infected with HSV (adsorbed multiplicity of 10 PFU/cell). After 90 min for adsorption, the virus was removed and 1 ml of medium containing [^{14}C]glucosamine (1 μCi/ml) or [^{35}S]methionine (20 μCi/ml) was added. The radioactive medium added to half the samples contained TM at 2 μg/ml. At the indicated times, the acid-precipitable radioactivity was determined in duplicate or triplicate cover slips.* (○) *Infected control;* (●) *uninfected control;* (□) *infected, TM treated;* (■) *uninfected, TM treated.*

also found. The immune precipitate formed with extracts of TM-treated cells contained distinct polypeptides with molecular weights of 50K, 85K, and 110K (Fig. 3, lane D), as well as less-distinct components with molecular weights of about 70K and 100K.

To confirm the effect of TM on glycosylation, [2-^{3}H]mannose was employed as a specific carbohydrate label (9). Figure 3 shows that during a pulse and subsequent chase, mannose was incorporated into polypeptides with molecular weights of 52K, 110K, and 120K in lane J and 59K, 120K, and 130K in lane K. Each of these polypeptides was immunoprecipitated by anti-ENV-1 serum (lanes E and F). These results are in accord with earlier results (9). Mannose-labeled proteins were not immunoprecipitated from extracts of TM-treated cells (lanes G and H), although several proteins labeled with arginine were precipitated under the same conditions (lane D). Long-term exposure of the fluorogram failed to reveal any mannose label in the immune precipitates from the TM-treated cell extract. The radioactivity in the immunoprecipitated bands shown in lanes J and K, respectively, was: 52K, 3,470 and 270 cpm; 59K, 306 and 1,260 cpm; 110K, 5,360 and 250 cpm; 120K, 2,070 and 2,000 cpm; 130K, 1,200 and 4,620 cpm. Comparable areas from the TM-treated extract (lanes G and H) contained a background level of radioactivity (60 cpm), as did the areas corresponding to the 50K and 85K polypeptides.

The kinetics of glycoprotein synthesis were followed by means of [^{35}S]methionine incorporation and precipitation with anti-ENV-1 serum. Infected cells with or without TM treatment were pulsed with [^{35}S]methionine for 15 or 30 min at 5 h postinfection, and the radioactive polypeptides in these cells were analyzed alongside extracts from cells that had subsequently been chased for 5 h. Radioactive bands at 52K, 110K, and 120K were observed in immune precipitates from cells labeled for 15 min with [^{35}S]methionine (Fig. 4, lane B). These bands increased in intensity after 30 min of labeling (Fig. 4, lane C). As was observed (9; Fig. 3), the 52K polypeptide was replaced during the chase by a 59K molecule (gD), the 110K polypeptide disappeared, and the radioactivity incorporated into the 130K region (gC) increased (Fig. 4, lane D).

In contrast, the immune precipitates formed with extracts of TM-treated cells gave prominent bands at 50K, 85K, and 110K (Fig. 4, lanes F, G, and H). The intensity of these bands increased between 15 and 30 min and decreased during the chase period. Control immune precipitations carried out with an extract from uninfected cells (lane J) or with a preimmune serum confirm the specificity of the anti-ENV-1 serum

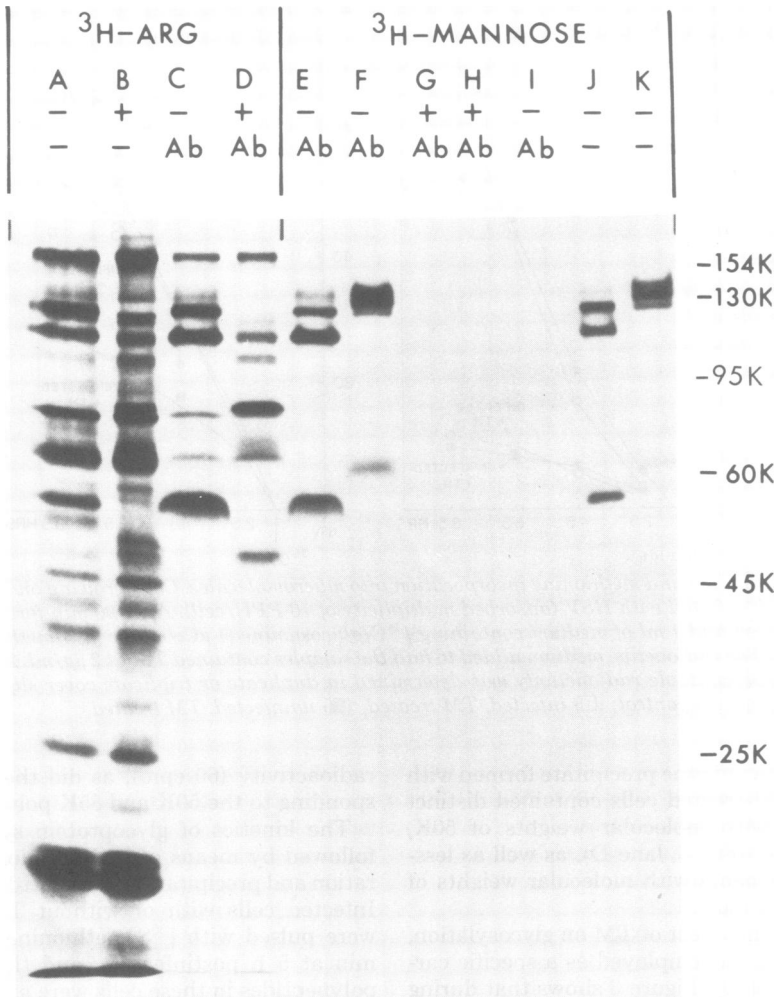

FIG. 3. *Immune precipitation of [^{3}H]arginine and [^{3}H]mannose-labeled polypeptides. HSV-infected cells with or without TM were labeled 5 h after infection with mannose or arginine. Extracts were prepared after a 30-min pulse or after a 30-min pulse followed by a 5-h chase. After reaction with anti-ENV-1 serum and Staphylococcus A protein, the precipitates were dissociated and electrophoresed, and the radioactive bands were detected by fluorography. Lanes A, B, C, and D show the [^{3}H]arginine polypeptides. Lanes E, F, G, H, I, J, and K show extracts from cells labeled with [^{3}H]mannose. Complete extracts were placed in lanes A, B, J, and K. The remainder of the lanes contain immune precipitates as indicated by the heading Ab. Extracts and precipitates from TM-treated cells (lanes B, D, G, and H) are designated with a + sign. The extracts from cells labeled with mannose and chased for 5 h are shown in lanes F and K. Lane I shows the uninfected cell extract.*

(lanes K and L). The radioactivity in selected bands was determined by cutting appropriate sections from the gel and determining the amount of radioactivity by scintillation counting. These data (Table 1) confirm the qualitative impressions given by the radioautograph that the antigenic polypeptides synthesized during the 30-min pulse in the presence of TM decrease in amount during the chase period, with about 30 to 40% of the labeled material remaining in the 50K, 85K, and 110K polypeptides after 5 h (Table 1).

Tryptic peptide analysis. To obtain information on the structural relationships between the antigenic polypeptides synthesized in the presence and absence of TM, the polypeptides were eluted from gels and digested with trypsin, and the tryptic peptides obtained were compared. The peptide patterns (Fig. 5) for the 50K polypeptide and pgD(52) labeled with [^{35}S]me-

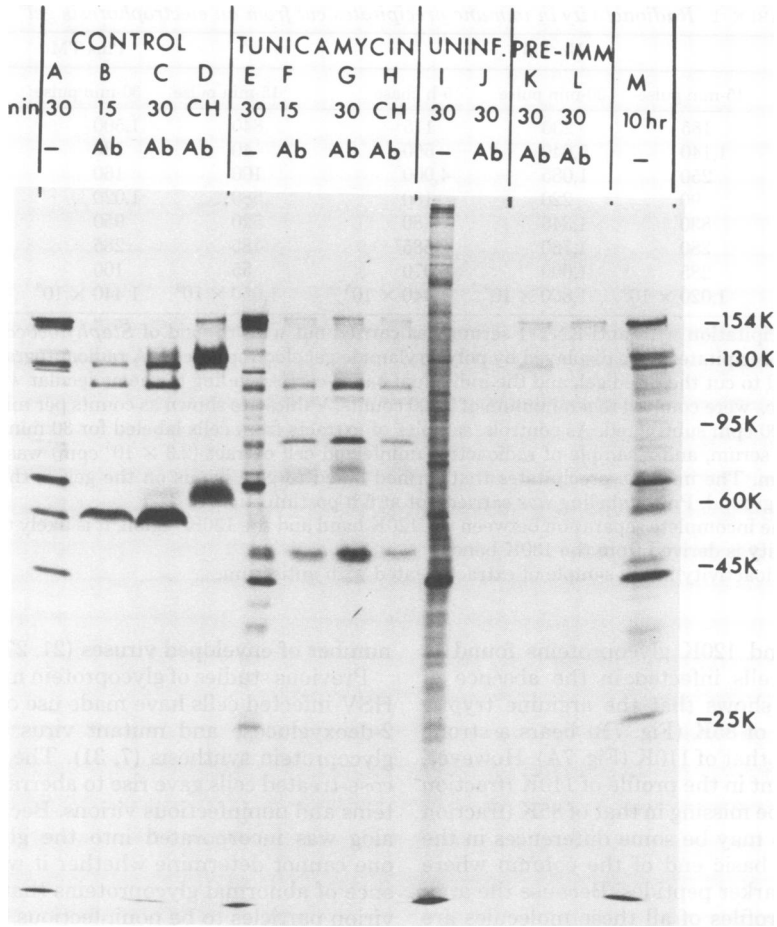

FIG. 4. *Immune precipitation of [^{35}S]methionine-labeled polypeptides. Infected cells were labeled with 100 μCi of [^{35}S]methionine for 15 (lanes B and F) or 30 min (lanes A, C, E, and G). Lanes D and H show 5-h chases of the samples labeled for 30 min and are designated with the heading CH. The immune precipitates obtained with anti-ENV-1 serum are shown in lanes B, C, D, F, G, H, and J, as indicated by the heading Ab. Extracts from TM-treated cells were used to obtain the precipitates shown in lanes F, G, and H. Lanes A and E show the complete extract from the control and TM-treated cells labeled for 30 min, and lanes K and L show the immune precipitates produced when these extracts were reacted with preimmune serum. Lane M shows a complete extract of infected cells labeled from 5 to 15 h.*

thionine were quite similar. In both cases, a single methionine-labeled peptide eluted at a characteristic pH (pH 3.33) and position relative to the peptides of the internal marker. As previously reported for pgD(52) (9), more than two-thirds of the radioactivity in the 50K digest was not retained by the column. Since the methionine peptide profiles were relatively simple, we also examined the arginine peptides of the 50K and pgD(52). The peptide patterns obtained from the 50K and pgD(52) polypeptides labeled with [^{3}H]arginine were similar (Fig. 6). On both chromatograms, two groups of four and six peptides are distinguishable. Three of the first four peptides elute at similar pH values, whereas the fourth peptide from the 50K polypeptide elutes at a somewhat higher pH value than its counterpart in pgD(52). The second group of six peptides also show similarities but elute in somewhat different positions relative to the internal markers. The methionine and arginine peptide patterns taken together indicate a high degree of homology between the 50K and 52K polypeptides. It should be noted that the same methionine and arginine peptide profiles were obtained for pgD(52) grown in either BHK or KB cells. In an effort to determine which of the glycoproteins in the 130K region is related to the 85K polypeptide found in TM-treated cells, we compared the arginine peptides of the 85K to those

TABLE 1. *Radioactivity in immune precipitates cut from an electrophoresis gel*[a]

Gel band	No TM			Plus TM		
	15-min pulse	30-min pulse	5-h chase	15-min pulse	30-min pulse	5-h chase
50K	185	230	115	840	1,500	508
52K	1,140	5,840	560	40	130	50
59K	250	1,085	4,000	100	160	145
85K	90	220	180	580	1,020	340
110K	830	1,340	80	520	950	290
120K	280	1,760	2,585[b]	185	285	40
130K	285	1,000	6,070	55	100	0
Total cpm[c]	$1,020 \times 10^3$	$1,860 \times 10^3$	$1,440 \times 10^3$	$1,050 \times 10^3$	$1,440 \times 10^3$	$1,020 \times 10^3$

[a] Immune precipitation with anti-ENV-1 serum was carried out with the aid of *Staphylococcus* A protein, and the washed precipitates were displayed by polyacrylamide gel electrophoresis. A radioautograph analogous to Fig. 4 was used to cut the dried gel, and the individual bands corresponding to the molecular weights shown, e.g., 50K, 85K, etc., were counted to a minimum of 5,000 counts. Values are shown as counts per minute with the background of 200 cpm subtracted. As controls, samples of extracts from cells labeled for 30 min were reacted with preimmune serum, and a sample of radioactive uninfected cell extract (2.3×10^6 cpm) was reacted with anti-ENV-1 serum. The immune precipitates that formed failed to give bands on the gels with radioactivity higher than background. Pulse-labeling was carried out at 5 h postinfection.

[b] Because of the incomplete separation between the 120K band and the 130K region, it is likely that a fraction of this radioactivity is derived from the 130K band.

[c] The total radioactivity in the sample of extract treated with antiserum.

of the 110K and 120K glycoproteins found in pulse-labeled cells infected in the absence of TM. Figure 7 shows that the arginine tryptic peptide profile of 85K (Fig. 7B) bears a strong resemblance to that of 110K (Fig. 7A). However, a peptide present in the profile of 110K (fraction 85) appears to be missing in that of 85K (fraction 75). There also may be some differences in the profiles at the basic end of the column where there are no marker peptides. Because the arginine peptide profiles of all these molecules are very complex, double-label experiments must be done before further conclusions can be drawn.

DISCUSSION

In this report we show that BHK cells fail to produce infectious particles in the presence of TM. At the concentration of TM employed, viral glycoprotein synthesis was specifically inhibited. This result is consistent with the known inhibitory effect of TM on attachment of *N*-acetyl glucosamine to a lipid carrier (dolichol phosphate), which in turn blocks core glycosylation of glycoproteins in eucaryotic cells (36). We infer from our experiments with TM that the synthesis of HSV glycoproteins involves the use of cellular enzymes for glycosylation and that HSV proteins produced in the presence of TM essentially lack carbohydrate side chains. The effect of TM on infectivity suggests that glycoprotein synthesis is required for production of infectious virus, a phenomenon previously reported for a number of enveloped viruses (21, 27, 32, 40).

Previous studies of glycoprotein maturation in HSV-infected cells have made use of the analog 2-deoxyglucose and mutant virus defective in glycoprotein synthesis (7, 31). The 2-deoxyglucose-treated cells gave rise to aberrant glycoproteins and noninfectious virions. Because the analog was incorporated into the glycoproteins, one cannot determine whether it was the presence of abnormal glycoproteins that caused the virion particles to be noninfectious. Assessment of the importance of the glycoprotein in infection is more difficult in the temperature-sensitive glycoprotein-deficient mutant because at the nonpermissive temperature DNA synthesis did not occur, and other changes took place within the infected cell. The effects of TM on HSV replication constitute a third situation in which infectious virus is not produced and viral glycoprotein synthesis is specifically inhibited.

By use of specific antisera, we have shown that the glycosylated precursors to viral glycoproteins normally found in pulse-labeled infected-cell extracts were replaced in TM-treated cells by lower-molecular-weight polypeptides. Tryptic peptide analysis of these polypeptides revealed that the 50K molecule formed in the presence of TM is structurally similar to the normal precursor of glycoprotein D, pgD(52), and that the 85K molecule synthesized in the presence of TM is structurally similar to the normal 110K glycoprotein precursor.

The unglycosylated analog of HSV glycoproteins may be unstable, since the amount of ra-

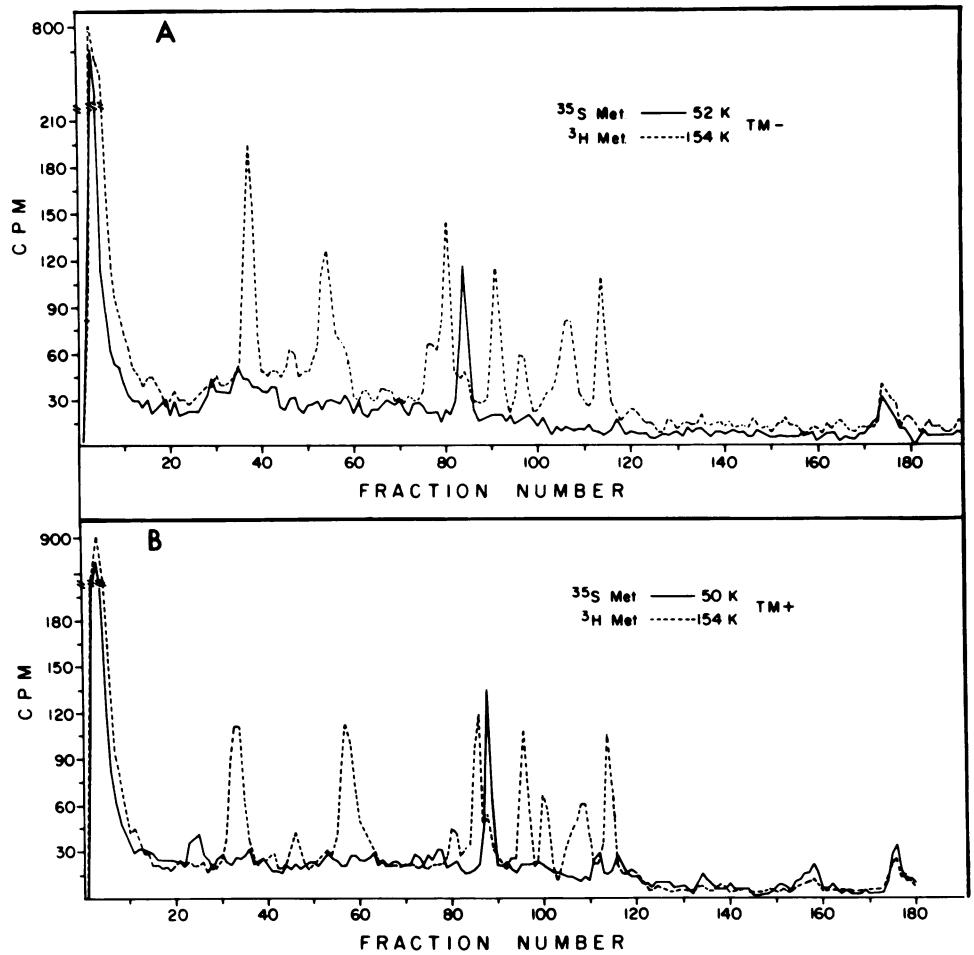

FIG. 5. *Tryptic fingerprint analyses of [^{35}S]methionine-labeled HSV glycoprotein precursor pD (52K) and the 50K polypeptide. (A) 52K (pD) (solid line), isolated by immunoprecipitation and sodium dodecyl sulfate-polyacrylamide gel electrophoresis from [^{35}S]methionine pulse-labeled extracts (30 min, 5 h after infection), was codigested and cochromatographed on a column of Chromobeads P with [^{3}H]methionine-labeled 154K capsid polypeptide (broken line) isolated in an analogous way. (B) The 50K polypeptide (solid line), isolated by immunoprecipitation and sodium dodecyl sulfate-polyacrylamide gel electrophoresis from [^{35}S]methionine pulse-labeled TM-treated cell extract, was codigested and cochromatographed with the [^{3}H]methionine-labeled 154K capsid polypeptide as internal marker.*

dioactivity that was found in these molecules decreased during the chase. This result suggests that HSV glycoproteins may behave like influenza virus glycoproteins (32) and fibronectin (25), which are more susceptible to degradation when unglycosylated. Thus, carbohydrate side chains normally added to polypeptides may modulate proteolytic reactions that accompany glycoprotein synthesis (1). Alternatively, these results could be explained if the unglycosylated polypeptides were inserted into the envelope of noninfectious virions (12, 28) and released from the cell, or if they were aggregated and failed to be solubilized by the detergent used to lyse the cells (13). Electron microscopy of TM-treated HSV-infected cells revealed the presence of enveloped particles (L. Pizer, data not shown). To determine whether these particles contain the 50K or 85K polypeptides or both will require a study of these components in purified virion preparations. In addition, direct microscopic observation showed that HSV-infected cells treated with TM failed to undergo fusion (L. Pizer, unpublished data), confirming earlier work that glycoproteins are needed for syncytium formation (10, 17, 20, 22).

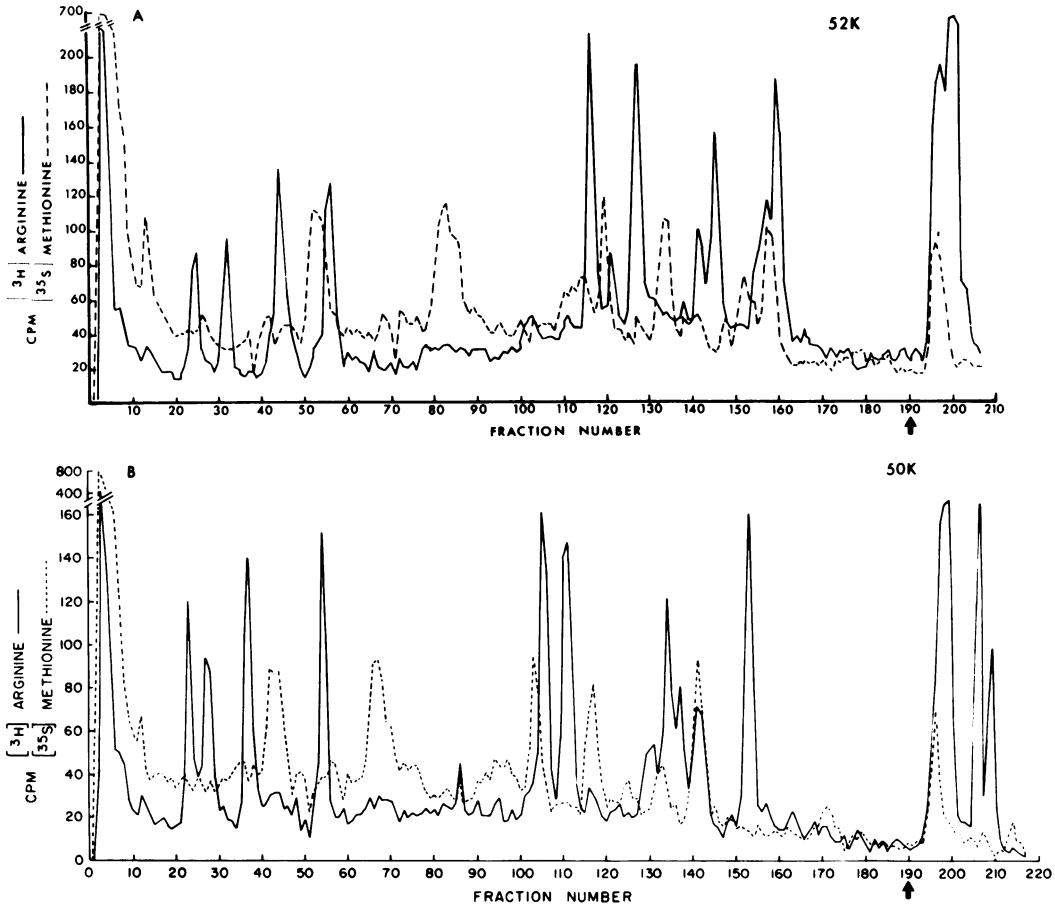

FIG. 6. *Tryptic fingerprint analyses of [^{3}H]arginine-labeled 52K and 50K polypeptides. Immune precipitates of [^{3}H]arginine-labeled 50 and 52K polypeptides (analogous to lanes C and D in Fig. 3) were isolated from sodium dodecyl sulfate-polyacrylamide gels, digested and cochromatographed with [^{35}S]methionine-labeled 154K polypeptide. The patterns obtained with the 52K band and the 50K band are shown in chromatograms A and B, respectively.*

The kinetics of labeling are compatible with the possibility that the 50K and 85K polypeptides could be normal unglycosylated precursors to viral glycoproteins which only accumulate in detectable amounts in pulse-labeled TM-treated cells. However, because of the data with vesicular stomatitis virus (28), which indicate that glycosylation of polypeptides proceeds while the nascent chains are elongating, and because of our previous experiments, which showed glycosylated precursors in HSV-infected cells after a short pulse time (9), we believe that the 50K and 85K are not normally present in HSV-infected cells as precursors but are polypeptides with amino acid sequences and antigenic determinants similar to the viral glycoproteins. Furthermore, the fact that neither the 50K nor the 85K polypeptide is identical in tryptic peptides to the analogous glycosylated form could be due to proteolysis or other alterations that occur to these molecules in TM-treated cells (12). Therefore the size of the TM polypeptides may not be an accurate reflection of the size of the normal glycoprotein minus its carbohydrate component. In most cases studied so far, however, the size of the polypeptide found in the presence of TM agreed with the calculation of the size of the core protein minus the oligosaccharide as calculated by chemical methods (6, 24). We had previously shown that the molecular weight of the core oligosaccharide of pgD(52) was 1,800. From other experiments we concluded there were probably more than one oligosaccharide chain attached to gD. Thus, if the 50K peptide formed in the presence of TM represents most if not all of the amino acids in gD, then it is likely that pgD(52) contains between one and three mannose-rich core oligosaccharides. Using the same

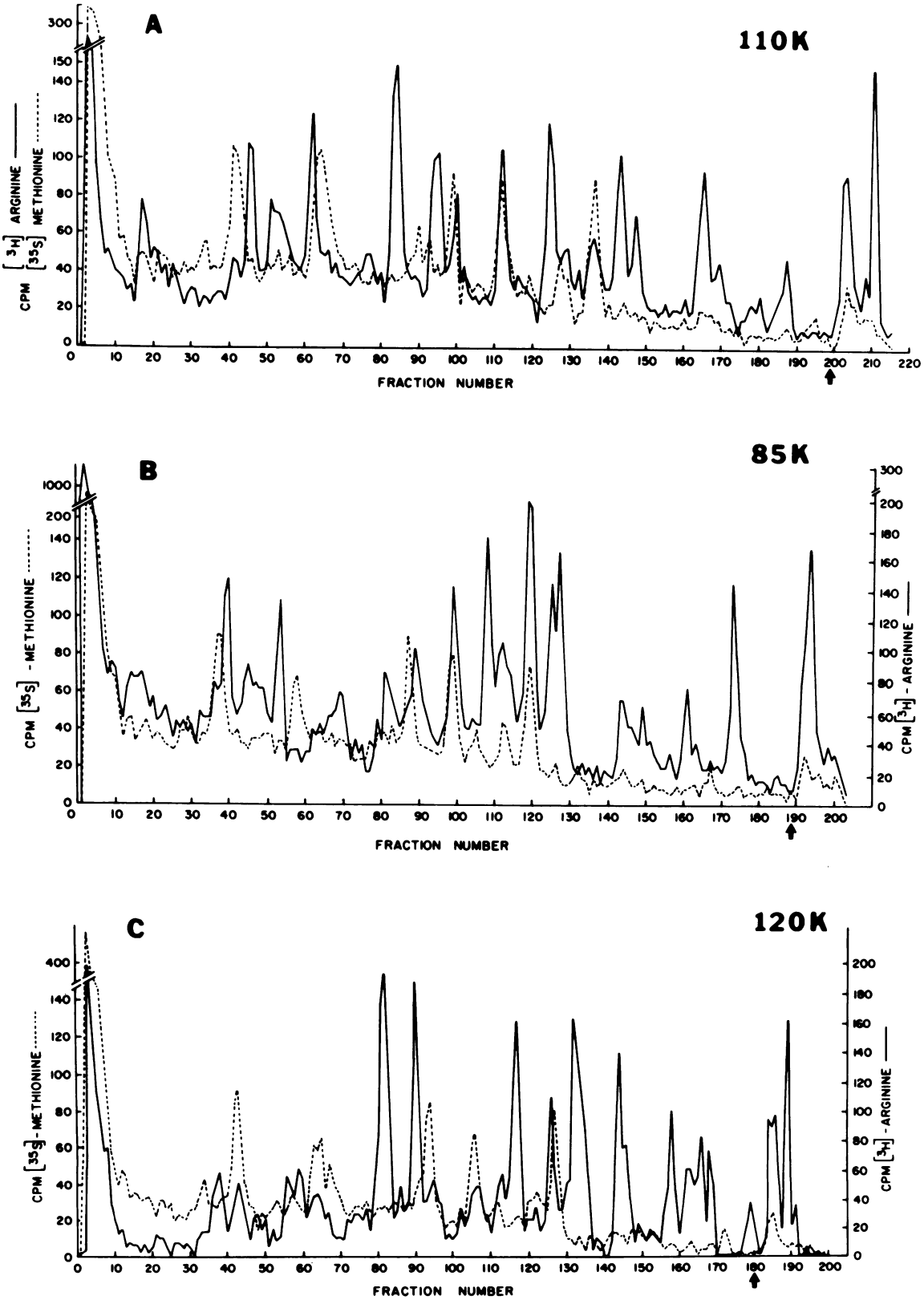

FIG. 7. *Tryptic fingerprint analyses of [^{3}H]arginine-labeled 85K, 110K, and 120K polypeptides. The 85K band was isolated by immune precipitation and sodium dodecyl sulfate-polyacrylamide gel electrophoresis from a TM-treated extract of cells labeled for 30 min with [^{3}H]arginine as described in the legend to Fig. 3. The 110K and 120K bands were isolated in a similar manner from the extract of control cells. [^{35}S]methionine-labeled 154K polypeptide was employed as an internal marker for chromatography.*

assumptions for the 110K glycoprotein, it is possible that this molecule may contain as many as 10 to 15 oligosaccharide chains. Further studies will be necessary to validate these calculations.

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