JOURNAL OF VIROLOGY, Mar. 1979, p. 1149-1158
0022-538X/79/03-1149/10$02.00/0

Vol. 29, No. 3

Membrane Proteins Specified by Herpes Simplex Viruses

III. Role of Glycoprotein VP7(B_2) in Virion Infectivity

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Received for publication 25 August 1978

Experiments done with a temperature-sensitive mutant of herpes simplex virus type 1 (HSV-1) have revealed that one of the virion glycoproteins, designated VP7(B_2), is apparently not required for the production of enveloped virus particles, whereas it does play a critical role in virion infectivity. The mutant, designated HSV-1[HFEM]tsB5, fails to accumulate VP7(B_2) at nonpermissive temperature and produces virions that lack detectable quantities of this glycoprotein and that have very low specific infectivity. The poor infectivity of the virions is most readily explained by failure of penetration into the host cell rather than by failure of adsorption to cells because it was shown that the VP7(B_2)-deficient virions can bind to cells and that polyethylene glycol, an agent known to promote membrane fusion, can significantly enhance infectivity of the adsorbed virions.

The lipid-containing envelope of herpes simplex virus (HSV) mediates entry of the virus into a host cell, based on findings that naked nucleocapsids or particles with disrupted envelopes have very low specific infectivities relative to intact virions (22, 30) and that envelope constituents are targets of neutralizing antibodies (2, 8, 16, 18, 27). Although the precise mechanism by which the HSV envelope facilitates viral entry into a host cell is not understood, it seems likely that the first event in the infectious process is specific adsorption of the virion to the host cell surface and that proteins on the virion surface mediate this initial interaction with the cell. It has been proposed that, subsequent to adsorption, the virion enters the host cell either by phagocytosis (3), a process which need not require the activity of any virion constituent, or by fusion between the virion envelope and cell surface membrane (9, 14). This latter hypothesis implies that virion constituents may play an active role in viral entry of a host cell as well as in adsorption to the cell.

In investigating the mechanism by which HSV initiates infection, it seems reasonable to focus initially on the functions of the envelope glycoproteins, the major species of which have been designated VP8(C_2), VP7(B_2), VP8.5(A), and VP18(D_2) in HSV type 1 (HSV-1) (6, 28), because these proteins are known to be exposed at the surfaces of intact virions (15, 19; Sarmiento and Spear, manuscript in preparation). Experiments were carried out with a temperature-sensitive (ts) mutant of HSV-1 isolated by Alexander Buchan (University of Birmingham, Birmingham, England) that has a selective defect in the accumulation of glycoprotein VP7(B_2) at nonpermissive temperature. Previous studies (13) performed with this mutant, and with ts recombinants obtained by crossing the mutant with another HSV-1 strain, indicated that temperature sensitivity of VP7(B_2) accumulation correlated with temperature sensitivity of infectious virus production and of HSV-induced cell fusion. We report here that virions can be produced at nonpermissive temperature in the absence of VP7(B_2) and that these VP7(B_2)-deficient virions have very low specific infectivity. Our results also indicate that VP7(B_2) is required for the penetration stage of the infective process, subsequent to adsorption.

MATERIALS AND METHODS

Cells. African green monkey cells (Vero) and HEp-2 cells were grown in monolayer cultures in Dulbecco modification of Eagle minimal essential medium (DME), supplemented with 10% fetal calf serum (both medium and serum were obtained from KC Biologicals, Inc., Lenexa, Kans.). Cell stocks were maintained in antibiotic-free medium, but penicillin and streptomycin were added to the cultures used for experiments. The Vero cells were used for titrations of infectious virus (20), and HEp-2 cells were used for most other purposes.

Virus strains. A ts mutant, designated HSV-1[HFEM]tsB5, and its non-temperature-sensitive parent HSV-1[HFEM] were obtained from Alexander

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Buchan. HSV-1[HFEM]tsB5 was isolated after bromodeoxyuridine mutagenesis and has a DNA^+ phenotype (A. Buchan, personal communication). Certain other properties of this mutant have been described in a previous publication (13). Virus stocks were produced in HEp-2 cells as previously described (21), with infection carried out at low input multiplicities of virus (0.01 PFU/cell) to minimize the production of defective viruses.

Chemicals. New England Nuclear Corp. was the supplier of L-[^{35}S]methionine (100 to 400 Ci/mmol) and D-[1-^{14}C]glucosamine hydrochloride (45 to 55 mCi/mmol). The chemicals used for acrylamide gel electrophoresis were all purchased from Bio-Rad Laboratories (Richmond, Calif.). Dextran T10 is a product of Pharmacia Fine Chemicals AB (Uppsala, Sweden), and the polyethylene glycol (PEG) 6000 used in experiments described here was a product of J. T. Baker Chemical Co. (Phillipsburg, N.J.).

Electron microscopy. HEp-2 cells (8×10^6 cells per culture) were infected at 3 PFU/cell with HSV-1[HFEM] or with HSV-1[HFEM]tsB5 and maintained at 34 or 39°C. At 4 and 18 h after infection the cultures were subjected to cycles of freezing and thawing, for the titration of infectious virus. At 3 and 14 h after infection, other cultures were washed with phosphate-buffered saline and then fixed with 2% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2). The fixative remained in contact with the cells for 0.5 h at 4°C, and then the cells were scraped from the glass surface, collected by centrifugation, postfixed for 0.5 h with osmium tetroxide in 0.067 M *s*-collidine buffer (pH 7.4), dehydrated in a graded series of ethanol washes, and embedded in Epon resin. Thin sections of the embedded samples were stained with lead citrate and uranyl acetate prior to examination. This procedure for the preparation of material for electron microscopy was adapted from one published by Sabatini et al. (24).

Purification of virions. Virions were purified from cytoplasmic extracts of infected cells essentially as described by Spear and Roizman (29) and modified by Heine et al. (6). Briefly, HEp-2 cell monolayers in 32-ounce (ca. 909-ml) bottles were infected with virus at a multiplicity of 3 PFU/cell and were maintained in medium 199 supplemented with 1% fetal calf serum (199-V). Incubation was for 18 h at 34 or 39°C. Radioactive precursors were added to the cells at 5 h postinfection in 199-V containing one-half the usual concentration of glucose plus [^{14}C]glucosamine at 0.75 μCi/ml or in 199-V containing 1/10 the usual concentration of methionine plus [^{35}S]methionine at 1 μCi/ml.

At the appropriate times after infection, the cells were scraped from the glass bottles into the culture medium and collected by centrifugation. For preparation of the cytoplasmic extracts, the infected cells were suspended in 1 mM sodium phosphate buffer, pH 8.0 (1 ml per 2×10^8 cells), and, after 10 min on ice, were disrupted in a Dounce homogenizer. The homogenization was carefully monitored by phase microscopy to ensure that most cells were disrupted with minimal nuclear breakage. Nuclear pellets, obtained after centrifugation of the homogenate for 10 min in a clinical centrifuge, were washed once with 1 mM phosphate buffer (1 ml per 2×10^8 nuclei). The cytoplasmic extract was combined with the nuclear wash, and samples (2 to 3 ml) were layered over 36-ml gradients of Dextran T10 (prepared in 1 mM phosphate buffer, pH 8.0, and ranging in density from 1.04 to 1.09 g/cm^3) and centrifuged for 1 h at 20,000 rpm in an SW27 rotor (4.3×10^6 *g*-min). Virions sedimented to a position near the middle of the tube and formed an opalescent band, which was collected from the gradient by aspiration into a syringe. Titrations for infectivity were performed immediately and/or the virions were frozen in the Dextran T10 solution and stored at −70°C until use; storage under these conditions caused no detectable loss of infectivity over a period of several months. Prior to electrophoresis the virion suspension in Dextran was thawed and diluted 1:5 with phosphate-buffered saline (PBS), and the virions were collected by centrifugation for 2 h at 5°C and 25,000 rpm in the SW27 rotor.

Electrophoresis of proteins on polyacrylamide gels containing SDS. Electrophoresis was performed on slabs of polyacrylamide (10 cm by 14 cm by 1.5 mm) containing 8.5% acrylamide. The procedure was essentially as described by Laemmli (12) except that *N',N'*-diallyltartardiamide was used as a cross-linker as described by Heine et al. (6). Some modifications were introduced to facilitate polymerization of the gels. Briefly, the acrylamide-*N',N'*-diallyltartardiamide solution was degassed for 3 min under vacuum, the concentrations of *N',N'*-diallyltartardiamide and ammonium persulfate were increased to 0.23 and 0.3%, respectively, in the main gel, and the concentrations of acrylamide and *N',N'*-diallyltartardiamide were increased to 6 and 0.15%, respectively, in the stacking gel. The combs used to form the sample wells were removed immediately after the stacking gel had polymerized (0.5 h) because it was found that delay in removal resulted in strong adherence of the gel to the comb and damage to the wells upon removal.

Samples for electrophoresis were dissolved in a concentrated dissociation buffer to yield final concentrations of the components as follows: 0.05 M Tris-hydrochloride (pH 7.0), 5% 2-mercaptoethanol, 2% sodium dodecyl sulfate (SDS), and a trace of bromophenol blue tracking dye. Samples with a high content of DNA (i.e., unfractionated infected cells) were sonically disrupted for 1 min. Except where otherwise noted, all samples were heated in a boiling water bath for 2 min just prior to electrophoresis. After electrophoresis the gels were fixed, stained with Coomassie brilliant blue, and destained, following the procedure of Fairbanks et al. (4), and were then dried and exposed to Cronex Medical X-ray film, which was processed in Kodak D-19 developer and Rapid Fix.

Quantitation of the adsorption of virions to Vero cells. Radiolabeled virions were purified from HEp-2 cells that had been infected with HSV-1[HFEM] or with HSV-1[HFEM]tsB5 and maintained either at 34 or at 39°C in the presence of medium containing [^{35}S]methionine, as described above. Replicate cultures of Vero cells (8×10^6 cells per culture) were exposed to 1-ml samples of the virion preparations, which had been appropriately diluted with PBS containing 0.1% glucose and 1% fetal calf serum (PBS-G-CS) so that all cultures received approximately the same number of virions. Adsorption

of the labeled virions to the cells was carried out at room temperature on a shaker. At 15, 30, 60, and 120 min after addition of the virion suspensions to the cells, the inocula were removed from duplicate cultures, the monolayers were washed twice with PBS, and then the cells were disrupted and detached from the plastic surface by dispersion with PBS containing 0.1% Nonidet P-40. To quantitate the radioactive virions that had attached to the Vero cells, the suspended contents of each culture flask were mixed with trichloroacetic acid, and the precipitates were collected on glass fiber filters, washed with trichloroacetic acid and ethanol, and dissolved in Soluene (Packard Instrument Co., Inc., Downers Grove, Ill.) in preparation for liquid scintillation spectrometry.

Enhancement of viral penetration by treatment of cells exposed to virus with PEG. Monolayer cultures of Vero cells were exposed to serial 10-fold dilutions of virus prepared in PBS-G-CS. After allowing 1 h for adsorption of virus, the inocula were removed, and the monolayers were washed with the supplemented PBS solution. The cultures were then carefully drained, and the cells were exposed to a solution (3 ml per culture) prepared by mixing 40 g of melted PEG 6000 with 36.4 ml of DME without serum. (The PEG was melted and sterilized by autoclaving and then cooled to 70°C before mixing with the DME.) Exposure of the cells to this PEG solution (after cooling to 39°C) was kept to a minimum by decanting the solution immediately after swirling it over the monolayers and by rapidly washing away the adherent viscous residue. To facilitate mixing of the residue with the wash solutions, the first wash contained 1 part of PEG and 3 parts of serum-free DME by weight, and the second wash contained 1 part of PEG and 7 parts of serum-free DME. The final three washes were done with DME containing 10% fetal calf serum.

After this treatment with PEG, the cultures were incubated with DME containing 10% fetal calf serum for 2.5 h at 37°C, to allow PEG-induced membrane fusion to occur. Incubation of the cultures was then continued for 48 h at 34°C in DME containing 5% fetal calf serum and 0.1% pooled human gamma globulin, at which time the cultures were stained and the foci of infected cells were counted. Control cultures were infected, washed, and treated exactly as described above except that PEG was omitted from all solutions.

This procedure for treatment of the infected cells with PEG was adapted from a protocol devised by Pontecorvo (17) and modified by C. Basilico, New York University (personal communication), for the promotion of cell-to-cell fusion. Under the conditions used in these experiments, however, PEG 6000 did not induce cell-to-cell fusion in the Vero cultures, based on microscopic comparisons of the treated and untreated monolayers after fixation and staining.

RESULTS

Glycoprotein accumulation in cells infected with the mutant and parental virus strains. The electropherograms shown in Fig. 1 illustrate the selective temperature sensitivity of glycoprotein VP7(B_2) accumulation in HEp-2 cells infected with HSV-1[HFEM]tsB5 and the absence of this temperature sensitivity in cells infected with the parental strain HSV-1-[HFEM]. Most other viral proteins and glycoproteins appeared to be synthesized in roughly comparable amounts by both virus strains at either 34 or 39°C. Reproducible differences in the profiles of glycoproteins made by both viruses at the two temperatures are indicated by open arrows in Fig. 1 and probably result from the greater accumulation at 34°C than at 39°C of partially glycosylated precursors to VP7(B_2), VP8(C_2), and VP18(D_2) (28). The asterisk marks the position of another glycopeptide whose accumulation appeared to be somewhat temperature sensitive in some, but not all, experiments (see also Fig. 3 and 4). Quantitative differences in the accumulation of other polypeptides were sometimes noted in comparisons of cells infected with HSV-1[HFEM]tsB5 at 34 and 39°C; we do not yet know whether any of these differences are related to the ts defect that results in failure of VP7(B_2) accumulation and in reduction of infectious virus production.

Effect of the HSV-1[HFEM]tsB5 mutation on envelopment. A. Buchan had previously determined that viral DNA and nucleocapsids were produced in cells infected with HSV-1[HFEM]tsB5 at nonpermissive temperature (personal communication). Because glycopro-

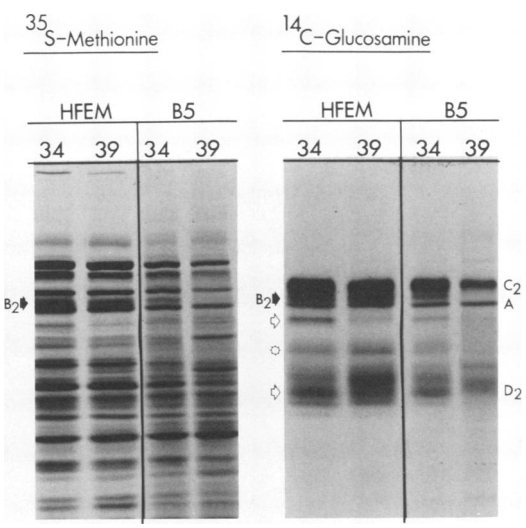

FIG. 1. *Electropherograms of polypeptides synthesized in HEp-2 cells infected with HSV-1[HFEM] or with HSV-1[HFEM]tsB5 and maintained either at 34°C or at 39°C. The cells were infected at a multiplicity of 20 PFU/cell, and radioactive precursors were present from 5 to 24 h after infection (1 μCi of [^{35}S]methionine per ml of 199-V containing 1/10 the usual concentration of methionine, or 0.75 μCi of [^{14}C]glucosamine per ml of medium 199-V containing 1/2 the usual concentration of glucose).*

tein VP7(B_2) is a structural component of the virion envelope, the possibility existed that envelopment of HSV-1[HFEM]tsB5 nucleocapsids might not occur at 39°C, providing a straightforward explanation for the temperature dependence of infectious virus production. To determine whether virions were produced at nonpermissive temperature, thin sections of infected cells were prepared and examined in the electron microscope. For this experiment HEp-2 cells were infected with HSV-1[HFEM]tsB5 or with HSV-1[HFEM] at 3 PFU/cell, and replicate cultures were maintained either at 34 or at 39°C. At 4 and 18 h after infection some of the cultures were harvested, and cell lysates were prepared to measure the yields of infectious virus; at 3 and 14 h after infection other cultures were harvested, and the cells were prepared for electron microscopy.

Examination of thin sections of the cells infected either with HSV-1[HFEM]tsB5 or with HSV-1[HFEM] and maintained at 34 or 39°C revealed that envelopment of both mutant and parental nucleocapsids occurred at either temperature. At 14 h after infection the budding of nucleocapsids was observed at the inner nuclear membrane, and enveloped particles could be found in the perinuclear space and in the cisternae of the endoplasmic reticulum. This is shown in Fig. 2 for cells infected with HSV-1[HFEM]-tsB5 and maintained at 39°C. Intracellular enveloped particles were not seen in any of the infected cultures that were processed for electron microscopy at 3 h after infection (micrographs not shown), indicating that the particles observed in Fig. 2 are progeny virions and not input virus. The production of virions in the cells infected with HSV-1[HFEM]tsB5 and maintained at 39°C was not accompanied by an increase in infectious virus, however, as is evident from the titrations that were performed on replicate cultures (Table 1).

Comparisons of HSV-1[HFEM]tsB5 virions produced at 34 and 39°C. Because cells infected with HSV-1[HFEM]tsB5 and maintained at 39°C fail to accumulate VP7(B_2) and yet produce virions, the question arose as to whether small amounts of VP7(B_2) might be

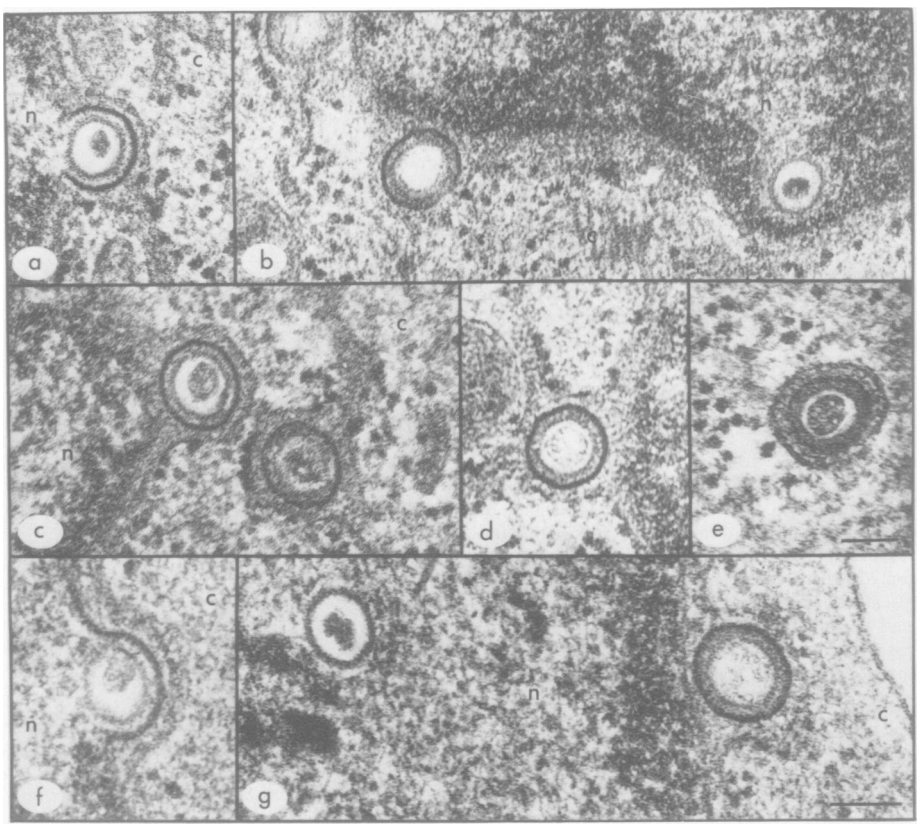

FIG. 2. *Electron micrographs of thin sections of HEp-2 cells infected with HSV-1[HFEM]tsB5 and maintained at 39°C for 14 h. (a–e) Magnification, ×40,000; (f and g) magnification, ×60,000. The bars represent 100 nm. n, Nucleus; c, cytoplasm.*

TABLE 1. *Infectious progeny produced in HEp-2 cells by HSV-1[HFEM] or HSV-1[HFEM]tsB5 at 34 or 39°C*

Virus	Hours postinfection	PFU per culture	
		34°C	39°C
HSV-1[HFEM]	4	3.2×10^4	3.3×10^4
	18	1.8×18^8	6.8×10^8
HSV-1[HFEM]tsB5	4	1.7×10^5	6.6×10^5
	18	5.0×10^8	2.0×10^5

made and sequestered into progeny virions. To determine whether the mutant virions produced at 39°C contained normal quantities of VP7(B_2), virions were purified from HEp-2 cells that had been infected with HSV-1[HFEM]tsB5 and then maintained at either 34 or 39°C in the presence of medium containing [^{35}S]methionine or [^{14}C]-glucosamine. It was found that the virions produced at 39°C lacked detectable quantities of labeled glycoprotein VP7(B_2) although all other structural proteins appeared to be present, as is shown by the comparisons presented in Fig. 3 and 4. It should be noted that HSV-1 virions contain a nonglycosylated or poorly glycosylated polypeptide designated VP7.5, which comigrates with VP7(B_2) but is differentiable from it (25) and which is probably responsible for the minor [^{35}S]methionine-labeled band detected in the position of VP7(B_2) in the virions produced at 39°C. Some differences were observed in the relative intensities of labeled bands formed by other virion polypeptides, but this has also been noted in comparisons of HSV-1[HFEM] virions produced at different temperatures (data not shown).

To compare the specific infectivities of HSV-1[HFEM]tsB5 virions produced at 34 and 39°C, samples of the virions purified from the cells maintained at the two different temperatures were assayed for infectious units and also were analyzed by electrophoresis on an SDS-acrylamide gel slab. After the separated proteins were stained with Coomassie brilliant blue, the gel was scanned in a densitometer to determine the relative amounts of the major capsid protein VP5 in each virion preparation. The relative quantities of protein VP5 provide a reliable estimate of the relative quantities of virions because, in both purified preparations, approximately 90% of the particles were enveloped as determined by electron microscopic examinations of negatively stained aliquots. The results (Table 2) demonstrate that the specific infectivity of the virions produced at 34°C was from 260-fold to 1,800-fold greater than that of the virions produced at 39°C. It should be noted that the quantity of purified virions obtained from cells maintained at 39°C was reproducibly less than could be obtained from an equivalent number of infected cells maintained at 34°C, but only by a factor of about three (Table 2). Therefore, the inability of HSV-1[HFEM]tsB5 to replicate at nonpermissive temperature is accounted for primarily by the low specific infectivity of the virions that are made at 39°C rather than by inhibition of virion production.

Comparisons of virions produced by the mutant and parental virus strains at 34°C. Results reported in the accompanying paper (25) reveal that VP7(B_2) specified by HSV-1[F] can be extracted from virions in the form of a dimer that is not completely dissociated by SDS plus 2-mercaptoethanol, except after heating. An experiment was done to determine whether the VP7(B_2) present in HSV-1[HFEM]tsB5 virions produced at 34°C differed from that of parental HSV-1[HFEM] virions with respect to its ability to form an SDS-stable dimer. Virions produced at 34°C were purified from HEp-2 cells infected with HSV-1[F], HSV-1[HFEM], or HSV-1[HFEM]tsB5 and were dissociated as usual for electrophoresis on SDS-acrylamide gels except that the samples were not heated prior to electrophoresis. The electropherogram shown in Fig. 4 reveals that a fraction of VP7(B_2) from HSV-1[F] and HSV-1[HFEM] virions was present in the form of an SDS-stable dimer, whereas this form of VP7(B_2) was not detected in the HSV-1[HFEM]tsB5 virions. This result demonstrates a physical difference between VP7(B_2) made by HSV-1[HFEM]tsB5 at 34°C and that made by the parental wild-type virus.

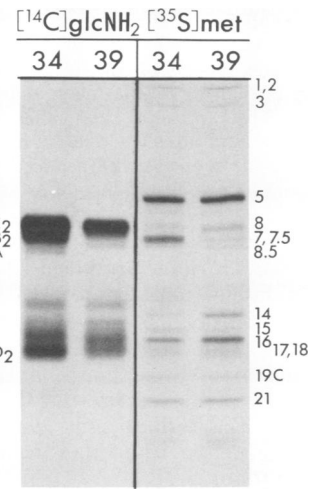

FIG. 3. *Electropherogram of polypeptides solubilized from virions produced at 34 or 39°C by HEp-2 cells infected with HSV-1[HFEM]tsB5. Incubation of the cells with radioactive precursors and purification of virions are described in the text.*

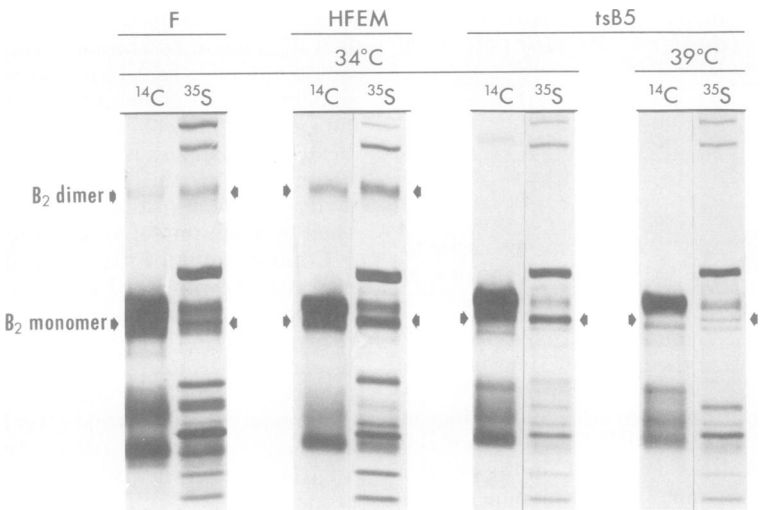

FIG. 4. *Electropherogram of polypeptides solubilized from virions produced at 34 or 39°C by HEp-2 cells infected with HSV-1[F], HSV-1[HFEM], or HSV-1[HFEM]tsB5. Heating of samples prior to electrophoresis was omitted.*

TABLE 2. *Relative specific infectivities of HSV-1[HFEM]tsB5 virions produced at 34 and 39°C*

Expt	Temp for virion production (°C)	Absorbance of protein VP5[a]	PFU/ml	Relative specific infectivity[b]
I	34	0.4	5.0×10^7	1.3×10^8
	39	0.15	1.1×10^4	7.3×10^4
II	34	0.5	1.8×10^8	3.6×10^8
	39	0.18	2.6×10^5	1.4×10^6

[a] Electron microscopic examination revealed that 80 to 90% of the virus particles present in each preparation were enveloped. Aliquots from each preparation were analyzed by electrophoresis on SDS-acrylamide gels, and the quantity of protein VP5 was estimated by densitometry of the gel after staining with Coomassie brilliant blue. Inasmuch as protein VP5 is the major capsid protein, this value, expressed in arbitrary units, indicates the relative number of virus particles present in each preparation.

[b] (PFU/milliliter)/absorbance of protein VP5.

To determine whether the infectivity of HSV-1[HFEM]tsB5 virions produced at 34°C was more heat labile than that of HSV-1[HFEM] virions, preparations of the two viruses were heated at 45°C for varying periods of time, and residual infectivity was then quantitated. The results (Fig. 5) demonstrate that the infectivity of HSV-1[HFEM]tsB5 virions was inactivated at a much higher rate than that of HSV-1[HFEM] virions.

Kinetics of adsorption of mutant and parental virions to Vero cells. Replicate monolayers of Vero cells were exposed to purified preparations of radiolabeled HSV-1[HFEM] or HSV-1[HFEM]tsB5 virions (produced at 34 and 39°C) for different periods of time and then washed extensively to remove unadsorbed virus, and the cells were processed for quantitation of bound radioactivity as described in Materials and Methods. The concentrations of virions in each of the three preparations used were adjusted so that approximately the same number of particles was added to each monolayer. The input multiplicities of infectious virus were approximately 2 PFU/cell for HSV-1[HFEM] produced at 34°C, 0.5 PFU/cell for HSV-1-[HFEM]tsB5 produced at 34°C, and 0.002 PFU/cell for HSV-1[HFEM]tsB5 produced at 39°C, reflecting the large difference in specific infectivities of the mutant virions produced at the two temperatures.

Virions in all three preparations could bind to Vero cells (Fig. 6). The kinetics of adsorption and percentage of input virus adsorbed were similar for HSV-1[HFEM] virions and HSV-1[HFEM]tsB5 virions produced at 34°C. The percentage of input virions adsorbed was somewhat lower, however, for the HSV-1[HFEM]-tsB5 virions made at 39°C, and the rate of adsorption appeared to decrease significantly between 1 and 2 h of incubation. Thus, the ratio of binding efficiencies (for HSV-1[HFEM]tsB5 produced at 34°C to that produced at 39°C) was only 1.3:1 after 30 min of incubation but was 2:1 after 2 h. It is doubtful that this difference in amount of virus adsorbed can account for the large difference in specific infectivities of the HSV-1[HFEM]tsB5 virions produced at 34 and 39°C (Table 2).

Use of PEG to enhance the infectivity of HSV-1[HFEM]tsB5 virions produced at

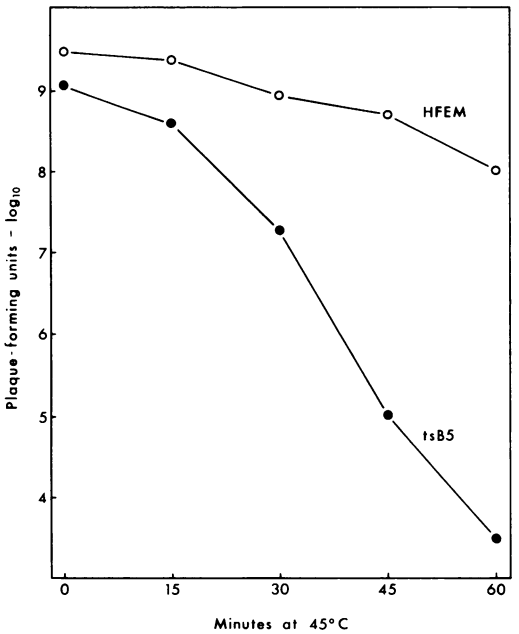

FIG. 5. *Loss of infectivity of HSV-1[HFEM] virions and HSV-1[HFEM]tsB5 virions due to incubation at 45°C. The virion preparations used were lysates of infected cells, and the quantitation of PFU was done on Vero cells.*

39°C. Agents that are capable of promoting membrane fusion, such as inactivated Sendai virus and PEG, have been used to promote the entry of retroviruses that are defective for penetration (10) or are unable to penetrate genetically resistant cells (5). To determine whether PEG could enhance the infectivity of VP7(B_2)-deficient HSV-1[HFEM]tsB5 virions produced at 39°C, the following experiment was done. Replicate cultures of Vero cells were exposed to various dilutions of HSV-1[HFEM] or HSV-1[HFEM]tsB5 virions produced at either 34 or 39°C. The virions used for infection were crude preparations (infected cell lysates) and also, in the case of HSV-1[HFEM]tsB5, purified preparations. After allowing 1 h for adsorption, the monolayers were extensively washed to remove unadsorbed virions and then briefly exposed to PEG 6000 solutions according to the procedure described in Materials and Methods. The numbers of foci of infected cells (plaques) were determined after incubation at 34°C for 48 h.

Treatment with PEG enhanced the infectivity of HSV-1[HFEM]tsB5 virions produced at 39°C by as much as 80-fold (Table 3). On the other hand, PEG treatment had no effect on the infectivities of HSV-1[HFEM] virions produced at either 34 or 39°C or of HSV-1[HFEM]tsB5 virions produced at 34°C. These results demonstrate not only that HSV-1[HFEM]tsB5 virions produced at 39°C have the capacity to bind to Vero cells but also that PEG treatment overcomes the block to their infectivity, for at least a fraction of the virions. It should be noted that the extent to which PEG enhanced the infectivity of mutant virions made at 39°C was variable and that the best results seemed to be obtained with the briefest exposure of the cells to the PEG-containing solutions. It should also be noted that, under the conditions used for these experiments, PEG 6000 did not induce cell-to-cell fusion in the Vero cell monolayers, as determined by microscopic examination of stained cultures.

DISCUSSION

The results of our studies with the viral mutant HSV-1[HFEM]tsB5 provide evidence that the envelope glycoprotein VP7(B_2) is not required for virion morphogenesis, at least not in the quantities usually present in wild-type infections, but is required for virion infectivity. The function of VP7(B_2) is apparently to promote the penetration of nucleocapsids into the host cell, probably by promoting fusion between the virion envelope and plasma membrane of the host cell.

Temperature-sensitive lesion in HSV-1[HFEM]tsB5. Several lines of evidence suggest that the failure of HSV-1[HFEM]tsB5 to replicate at nonpermissive temperature is directly related to the absence or deficiency of VP7(B_2) and, in addition, are consistent with the possibility that the ts mutation is actually in the structural gene for the VP7(B_2) polypeptide.

(i) Linkage of the conditional-lethal ts muta-

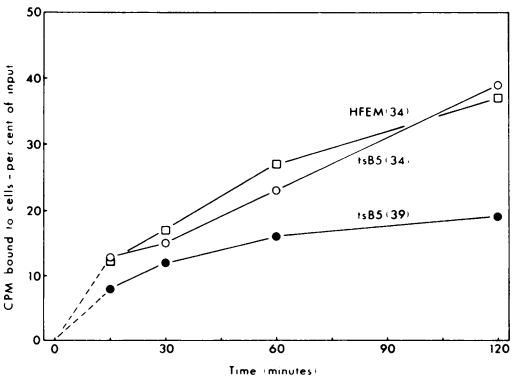

FIG. 6. *Binding of purified [^{35}S]methionine-labeled virions to monolayers of Vero cells. The HSV-1[HFEM]tsB5 virions were produced at either 34 or 39°C, and the HSV-1[HFEM] virions were produced at 34°C. Details of the experiment are given in the text.*

tion and of the failure to accumulate VP7(B_2) is evident from the results of genetic crosses and from analyses of a non-ts revertant (13). In this previous study HSV-1[HFEM]tsB5 was crossed with the non-ts strain HSV-1[MP], which has a syncytial marker (7, 11); all of the recombinant ts viruses isolated from six independent crosses failed to accumulate VP7(B_2) at nonpermissive temperature. Moreover, a non-ts revertant was isolated from one of these recombinants and was shown to produce normal quantities of VP7(B_2) at 39°C.

(ii) Linkage of the HSV-1[HFEM]tsB5 mutation and of the structural gene for VP7(B_2) is suggested by mapping studies which place both markers in the same region of the viral genome, from 0.30 to 0.45 map units (1, 23).

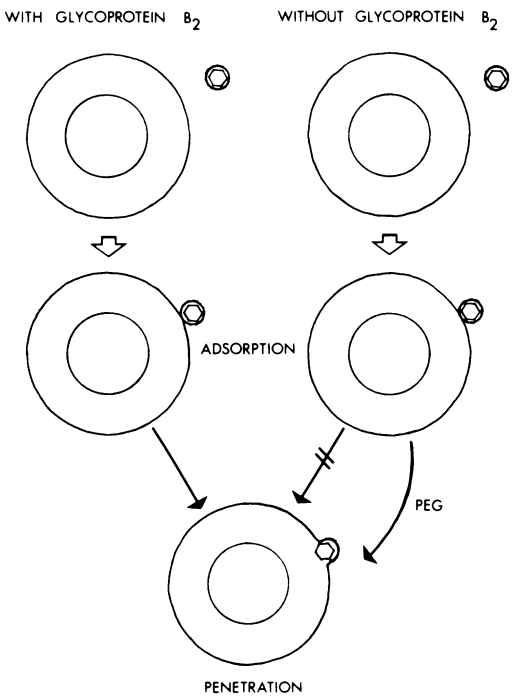

FIG. 7. *Representation of the probable defect in the infectivity of VP7(B_2)-deficient virions and of the postulated mode of viral penetration.*

(iii) It is also pertinent that VP7(B_2) made by HSV-1[HFEM]tsB5 at 34°C appears to be different from VP7(B_2) made by the parental virus, in that the mutant glycoprotein does not form an SDS-stable dimer of the kind produced by HSV-1[HFEM] (Fig. 4). This conformational difference could be due to mutation of the structural gene for the VP7(B_2) polypeptide, although our results do not rule out the possibility of mutation in a gene whose product is required for processing of the VP7(B_2) polypeptide. Whatever the reason for the conformational alteration of the mutant glycoprotein, it probably reflects a change in structure that could account for the heat lability of the virions produced at 34°C (Fig. 5) and for the failure of VP7(B_2) to accumulate at 39°C.

It is of interest that envelopment of HSV-1[HFEM]tsB5 nucleocapsids could occur at 39°C in spite of the greatly reduced quantities of glycoprotein VP7(B_2). On the basis of experiments reported here, however, we cannot rule out the possibility that small amounts of VP7(B_2) may be required for the envelopment of each nucleocapsid, particularly in light of findings that approximately ⅓ as many virions were recovered from cells maintained at 39°C as could be obtained from cells maintained at 34°C (Table 2). Another HSV-1 glycoprotein, designated VP8(C_2), is clearly not required for nucleocapsid envelopment because certain nonconditional nonlethal mutations result in the inability to produce VP8(C_2) (8, 13, 15). Virions lacking VP8(C_2) can be infectious, however, in contrast to our results obtained with the virions deficient in VP7(B_2). Recombinant viruses that fail to produce VP8(C_2) and are ts for VP7(B_2) production have been isolated (13), and it will be of interest to determine whether the envelopment of nucleocapsids can occur in the absence of both VP7(B_2) and VP8(C_2).

TABLE 3. *Effect of PEG on the infectivities of HSV-1[HFEM]tsB5 virions and HSV-1[HFEM] virions produced at 34 or 39°C*

Virus	Temp for virion production (°C)	PFU/ml		Ratio +PEG/−PEG
		−PEG	+PEG	
HSV-1[HFEM]	34	2.1×10^7	3.5×10^7	1.7
	39	1.2×10^8	1.1×10^8	0.9
HSV-1[HFEM]tsB5	34	2.1×10^7	2.6×10^7	1.2
	39	4.1×10^3	3.4×10^5	82.9
HSV-1[HFEM]tsB5 (purified virions)	34	3.1×10^7	2.0×10^7	0.6
	39	5.9×10^4	1.1×10^6	18.6

Role of VP7(B_2) in infectivity. The VP7(B_2)-deficient virions can evidently bind to cells (Fig. 6 and Table 3), indicating that their defect in infectivity is probably expressed subsequent to adsorption. Although the rate and extent of binding of VP7(B_2)-deficient virions appeared to be somewhat less than were observed with HSV-1[HFEM] virions or with HSV-1[HFEM]tsB5 virions produced at 34°C, these differences may be more apparent than real because, in the absence of penetration, the observed kinetics may reflect desorption as well as adsorption of particles, i.e., reversible binding of virions to cells.

The inability of the VP7(B_2)-deficient virions to initiate infection is apparently due to failure of penetration into the host cell because PEG, an agent that presumably acts at the cell surface, significantly enhanced the infectivity of the adsorbed mutant virions (Table 3). PEG is known to promote membrane fusion (17) and therefore may have induced penetration of the VP7(B_2)-deficient virions by promoting fusion between the virion envelope and plasma membrane of the cell, as depicted in Fig. 7. The results suggest that membrane fusion may be required for HSV penetration during the normal infective process, as was originally proposed by Morgan et al. (14), and that VP7(B_2) plays a role in this process. This postulated role of VP7(B_2) in virion infectivity is consistent with the previously reported finding that VP7(B_2) is required for the process of HSV-induced cell fusion (13). Whether the mechanism of membrane fusion between the virion envelope and host cell plasma membrane is the same as that responsible for cell-cell fusion is not known, but it seems likely that the two processes share features in common. Studies with other enveloped viruses known to promote cell-cell fusion have revealed that viral proteins responsible for this process are also required for virion infectivity (26, 31), so that there is precedent for relating these two phenomena.

It is probably pertinent to its function that wild-type VP7(B_2) can be extracted from virions in the form of a dimer, as is described more fully in the accompanying paper (25). Although the difference observed in the SDS-stable conformations of VP7(B_2) glycopolypeptides extracted from HSV-1[HFEM] and HSV-1[HFEM]tsB5 virions produced at 34°C appears not to reflect differences in the function of the glycoprotein, it should be noted that the results presented here provide no direct information about the similarities or differences of VP7(B_2) conformation in intact virions. Attention must now be focused on understanding the molecular interactions of VP7(B_2), the requirements for its function, and its precise role in promoting viral penetration.

ACKNOWLEDGMENTS

This work was supported by grant VC125 from the American Cancer Society. M.S. was a predoctoral fellow supported by Public Health Service training grant 5-T01-AI-00238 from the National Institutes of Health. M.H. is a predoctoral trainee supported by Public Health Service training grant 5-T32-GM-07183, and P.G.S. is the recipient of Research Career Development Award 5K04-CA00035, both grants from the National Institutes of Health.

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