JOURNAL OF VIROLOGY, June 1976, p. 1081-1087
Vol. 18, No. 3

Printed in U.S.A.

Preliminary Biochemical Characterization of the Factor(s) Responsible for Herpesvirus-Induced Exogenous Fusion

DEAN B. LEVITAN AND HERBERT A. BLOUGH*

Division of Biochemical Virology and Membrane Research, Scheie Eye Institute, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104

Received for publication 8 December 1975

Cell-free extracts prepared from herpes simplex virus-infected BHK-21 cells rapidly induced exogenous fusion when incubated with indicator monolayers of uninfected BHK-21 cells. Fusion was first observed at 1 h, and peak activity was reached by 4 h. Divalent cations were required for activity. Inhibition of indicator cell macromolecular synthesis, with metabolic inhibitors, failed to prevent formation of cell-free extract-induced polykaryocytes. Removal of virus particles from the cell-free extract by velocity sedimentation centrifugation did not affect cell-free extract exogenous fusion activity. Studies using molecular probes, namely, glycosidases, lectins, and antiserum (directed against either HSV envelope or capsid proteins), suggest that the factor(s) responsible for herpesvirus fusion is a fucosylated glycoprotein that is not a structural component of the virion.

A cytopathic effect frequently associated with the productive infection of cells by certain strains of herpes simplex virus (HSV) is the formation of large polykaryocytes (7, 19). The ability of HSV to produce syncytia is variable; e.g., some strains cause aggregation, whereas others cause rounding up of infected cells (25). Keller et al. (13) have attempted to relate the social behavior of HSV-infected cells in terms of viral-coded glycoproteins incorporated into host cell plasma membrane. Since viral-specified alterations in host cell plasma membrane architecture are essential for the induction of fusion by HSV, it is anticipated that expression of some portion of the HSV genome is required to fuse cells. In general, HSV-induced fusion is an endogenous event caused only by certain strains of this virus.

In contrast to HSV, strains of paramyxoviruses may induce either endogenous or exogenous fusion. The latter has been reported to occur exclusively with enveloped RNA viruses (14, 17). The characteristics of exogenous fusion are: (i) a direct interaction of plasma membrane with high-input multiplicities of virus particles occurs; (ii) viral genome expression is not required; and (iii) host cell macromolecular synthesis is unnecessary (3). The purpose of this study was to develop an in vitro system for producing exogenous fusion by HSV, comparable to paramyxovirus-induced exogenous fusion, to investigate the biological and biochemical parameters of this phenomenon. The system we report involves the rapid induction of exogenous fusion in indicator monolayers of normal BHK-21 cells by cell-free extracts (CL) prepared from HSV-infected BHK-21 cells. The results of this study suggest that HSV-induced fusion is mediated by a glycoprotein that is not a structural component of the virion. (This work was presented in part at the 75th Annual Meeting of the American Society for Microbiology, New York, N.Y., 27 April-2 May 1975.)

MATERIALS AND METHODS

Tissue culture. BHK-21 cells were used throughout. Cells were grown in monolayer cultures in Eagle minimal essential medium supplemented with 5% calf serum and appropriate antibiotics (9).

Virus stock and infectivity assay. "Seed" virus of HSV type 1, HF strain (a syncytia-producing strain), was propagated in BHK-21 cells (4). Viral infectivity was quantified by a modification of the plaque assay method of Flanagan (8).

Preparation of fusion-active CE. Fusion-active CE were prepared by infecting moderately confluent BHK-21 monolayers with HSV at an input multiplicity of 5 to 10 PFU/cell. Virus was adsorbed for 2 h at 37 C, after which fresh Eagle minimal essential medium supplemented with 5% calf serum and 2× (1 mM) arginine was added. The infection was allowed to proceed for approximately 13 h beyond the midpoint of the adsorption period, whereupon the cells were mechanically harvested with a rubber policeman, washed once with KIR (24), a biological salt solution low in divalent cations, sedimented by low-speed centrifugation, and suspended in hypotonic 50 mM Tris-hydrochloride buffer (pH 7.4). The cells were allowed to swell for 15 min and were then disrupted by 30 strokes of a tight-fitting Dounce homogenizer; isotonicity was restored by addition of 0.5 M sorbitol to a final concentration of approximately 10^7 cells/ml (hemo-

cytometer count) of 20 mM Tris-buffered 0.25 M sorbitol (TBS). The homogenate was then centrifuged for 10 min at 200 × *g* to sediment nuclei, cellular debris, and any remaining intact cells. The crude supernatant was then clarified by passage through a 1.2-μm membrane filter (Millipore Corp.); this filtrate possessed fusion activity and was used for all experiments.

Fusion experiments. Indicator BHK-21 cells were plated on 60-mm plastic dishes (Falcon Plastics, Oxnard, Calif.) in Eagle minimal essential medium with 5% calf serum at an initial density of 1.5×10^6 cells/plate the day prior to use; only lightly confluent monolayers were used to monitor fusion activity. Cultures were incubated at 37 C in 5% CO_2.

For all fusion experiments, the culture medium was removed and 0.9 ml of CE and 0.1 ml of 72 mM $CaCl_2$ in TBS were added directly to the indicator monolayers. The interaction of CE with cells was permitted to proceed for 4 h at 37 C (pH 7.4); the CE was then pipetted off, and the monolayers were washed once with cold phosphate-buffered saline (6), fixed with 95% methanol (vol/vol) and stained with Giemsa.

Fusion activity was quantified by the method of Nii and Kamahora (16) and was based on the average number of nuclei per polykaryocyte. Five or six high-powered fields (ca. 300 indicator cells), selected at random, were evaluated by light microscropy. All fusion indices represent the mean of duplicate or triplicate assays.

Antisera and infectivity neutralization assay. Immune sera directed against Nonidet P-40-solubilized envelope or capsid proteins were prepared in rabbits by the method of Watson et al. (25); this "anti-envelope" antiserum precipitated four of the major glycosylated polypeptides (p7, 8, 8.5, 18) of herpesvirus (23) and was a gift from Patricia Spear. The neutralization titers of these sera were determined by the plaque reduction method of Cohen and Wilcox (5). At this dilution, the anti-envelope protein antiserum reduced viral infectivity by ~90%; the anticapsid antiserum possessed negligible neutralization activity.

Velocity sedimentation centrifugation. Infectious virus particles were removed from the CE by velocity sedimentation centrifugation. Continuous gradients (36 ml) of 20 to 60% sucrose (wt/vol) in TBS were formed in cellulose nitrate tubes and precooled to 4 C. Fusion-active CE (1.5 to 2.0 ml) was then gently layered on top of the gradients with a Pasteur pipette, and the tubes were centrifuged for 2 h at 80,000 × *g* in an SW27 rotor using a Beckman model L2-65B ultracentrifuge. Fractions (2 ml) were collected by puncturing the bottom of each tube, dialyzed overnight in the cold against TBS, and assayed the next day for exogenous fusion activity as well as for infectious virus.

Chemicals. The dependence of CE-induced exogenous fusion on indicator cell macromolecular synthesis was investigated by exposing the indicator cells to various metabolic inhibitors. The inhibitors used were cycloheximide (10 μg/ml) (Nutritional Biochemicals, Cleveland, Ohio), actinomycin D (2 μg/ml) (Calbiochem, Los Angeles, Calif.), mitomycin C (10 μg/ml) (Sigma Chemical Co., St. Louis, Mo.), and cytosine arabinoside (3 μg/ml) (Aldrich Chemical Co., Milwaukee, Wis.). At the concentrations indicated, these drugs were found to inhibit by at least 95% the incorporation of appropriate isotopically labeled metabolic precursors into trichloroacetic acid-precipitable counts. The indicator monolayers were first pretreated for 3 h with one of the drugs, and the presence of the inhibitor was maintained throughout the 4-h period following the addition of CE to the indicator cells.

Concanavalin A, DNase (EC 3.1.4.6, bovine), β-galactosidase (EC 3.2.1.23, bovine), glucose oxidase (*Aspergillus niger*; EC 1.1.3.4), pyruvate kinase (EC 2.7.1.40, rabbit muscle), and lima bean trypsin inhibitor were purchased from Sigma Chemical Co., St. Louis, Mo.; α-L-fucosidase (EC 3.2.1.51, beef kidney) was from Boehringer Mannheim Co., New York, N.Y.; and RNase (EC 3.1.4.23, bovine) grade 1 was obtained from Gallard-Schlesinger, Carle Place, N.Y. Fucose-binding protein (*Ulex europeaus*) was obtained from Miles Laboratories, Kankakee, Ill.; crystalline trypsin (EC 3.4.21.4) was from Nutritional Biochemicals, Cleveland, Ohio; and wheat germ agglutinin was from Worthington Biochemicals, Freehold, N.J.

Coupling of enzymes to solid support. Commercially obtained enzymes were covalently linked to controlled-pore-size glass beads (Pierce Chemical Co., Rockford, Ill.) by amidization of an aliphatic side chain (terminating in an active *N*-hydroxy succinimide ester) to form a peptide linkage. The enzymes were first dialyzed against phosphate-buffered saline at pH 7.4 and then reacted at 4 C with 10 mg of glass beads/mg of enzyme protein. The coupling reaction was terminated after 3 h by addition of an equal volume of 2.0 M glycine. To assess the effect of enzymes of CE-induced exogenous fusion activity, the enzyme-glass bead complex was incubated for 1 h at 37 C with fusion-active CE. After the incubation period, the complex was sedimented by low-speed centrifugation to remove the enzyme; the CE supernatant was then removed and assayed for residual fusion activity as described above.

RESULTS

Kinetics of CE-induced fusion. The deeply stained and aggregated nuclei of large polykaryocytes resulting from 4-h incubation of fusion-active CE prepared from HSV-infected BHK-21 cells with an indicator monolayer are shown in Fig. 1A. Extracts similarly prepared from uninfected BHK-21 cells failed to cause fusion (Fig. 1B). Although not usually involving as many cells, CE-induced syncytia do appear to be morphologically quite similar to syncytia produced endogenously (Fig. 1C) 15 h after the productive infection of BHK-21 cells with HSV.

The onset and extent of fusion activity after the addition of CE to indicator cells is shown in Fig. 2. Fusion was first observed within 1 h after the addition of CE, with maximal activity

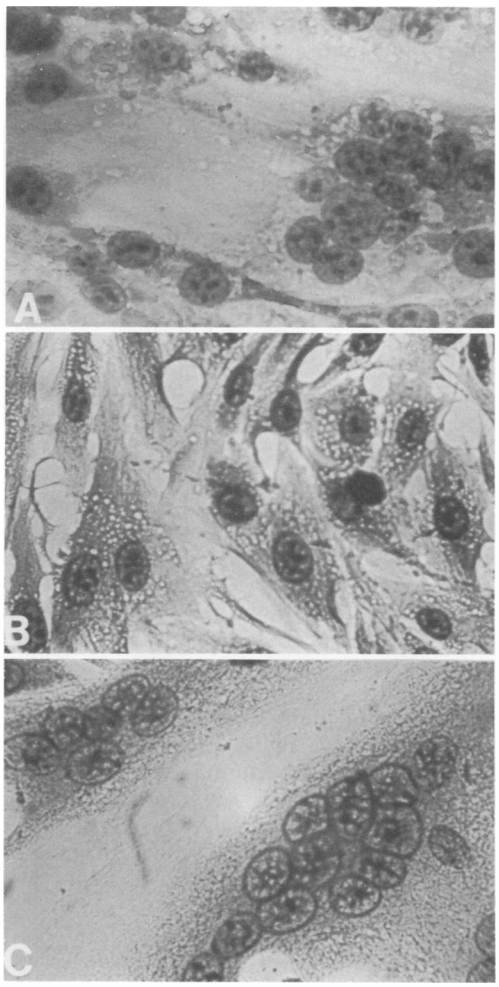

FIG. 1. *(A) Exogenous fusion of indicator BHK-21 cells induced by fusion-active CE prepared from HSV-infected BHK-21 cells. Indicator cells were incubated with CE for 4 h at 37 C and then fixed and stained. (B) Indicator cells similarly incubated with CE prepared from normal, uninfected BHK-21 cells did not fuse. (C) Endogenous induction of fusion 15 h after the productive infection of BHK-21 monolayers by HSV. All photomicrographs, ×320.*

seen by 4 h. Fusion indices of indicator cells incubated with control CE prepared from uninfected BHK-21 cells were identical to "background" levels characteristic of normal, uninfected BHK-21 cells grown in monolayers; i.e., a fusion index of 1.05 to 1.10 was noted. The time course of CE-induced fusion differed significantly from that of HSV-induced endogenous fusion in that, with the latter, syncytia were not detected until 7 h postinfection. Furthermore, the rapid onset of CE-induced fusion, i.e., its occurring well before the end of the HSV latent period, discounts the possibility that infectious virus present in the CE initiated a productive infection that led to endogenous fusion.

Inhibition of indicator cell macromolecular synthesis. That CE-induced fusion occurs exogenously is suggested not only by its rapid onset, but also by its occurring independently of indicator cell macromolecular synthesis. Drugs used to inhibit different stages of indicator cell metabolic functions (see Materials and Methods) had no effect on CE-fusion activity, thereby establishing that in this system the induction of fusion is an exogenous event (Table 1).

Divalent cation requirements of CE-induced exogenous fusion. Okada and Murayama (18) have shown a strict Ca^{2+} requirement for exogenous fusion of Ehrlich ascites tumor cells induced by Sendai virions; i.e., Mg^{2+} was unable to replace Ca^{2+}. Figure 3 illustrates that HSV-induced exogenous fusion has less specific divalent cation requirements in that exogenous fusion activity was stimulated to the same extent by Ca^{2+} or Mg^{2+} (as Cl^-) in the 1 to 10 mM range. When the divalent cation concentrations were <1 mM, the indicator cells detached from the culture dishes, and at concentrations >10 mM cytotoxicity was observed. Substitution of Mn^{2+} was less effective than either Ca^{2+} or Mg^{2+}.

Velocity sedimentation centrifugation of

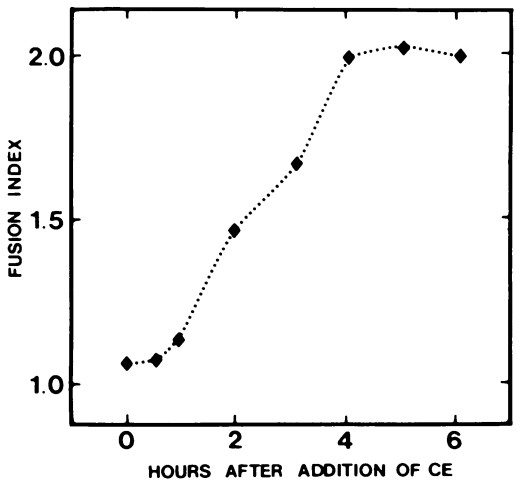

FIG. 2. *Onset and extent of CE-induced exogenous fusion. BHK-21 monolayers were incubated at 37 C with CE prepared from HSV-infected BHK-21 cells 13 h postinfection. At the indicated times, duplicate cultures were fixed and stained, and fusion indices were calculated. CE prepared identically from uninfected cells did not induce fusion.*

TABLE 1. *Effect of inhibition of indicator cell macromolecular synthesis on HSV-induced exogenous fusion activity*[a]

Indicator cell pretreatment[b]	Fusion index	% control
None (control)	1.94	100
Cycloheximide (10 μg/ml)	1.91	98.4
Actinomycin D (3 μg/ml)	2.02	104.1
Mitomycin C (10 μg/ml)	1.93	99.5
Cytosine arabinoside (30 μg/ml)	1.89	97.4

[a] Indicator monolayers were pretreated with an inhibitor for 3 h prior to addition of fusion-active HSV CE. The presence of the drug was maintained throughout the 4-h period following addition of the CE. The monolayers were then fixed and stained, and fusion indices were quantitated as described in Materials and Methods.

[b] When compared with controls, inhibitors block by 95% the incorporation of appropriate isotopically labeled precursors into trichloroacetic acid-precipitable counts.

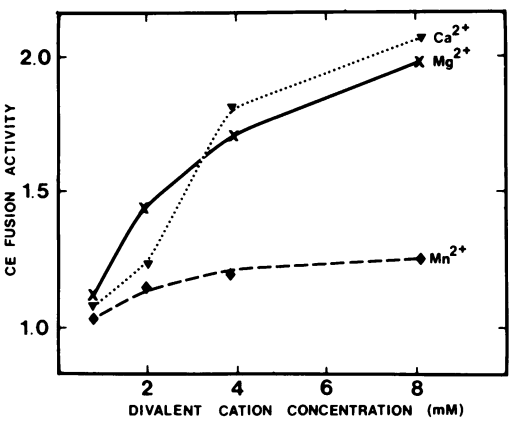

FIG. 3. *Dependence of CE fusion activity on divalent cation concentration. Fusion-active CE was assayed for exogenous fusion activity with the indicated concentration of a divalent cation: Ca^{2+} (▼); Mg^{2+} (×); Mn^{2+} (◆). After a 4-h interaction period, the indicator monolayers were fixed and stained, and fusion indices were calculated.*

fusion-active CE. To assess the role, if any, of HSV virions in CE-induced exogenous fusion, sucrose gradient velocity sedimentation centrifugation was used to remove virus particles from the CE (see Materials and Methods). As can be seen in Fig. 4, CE-associated infectious virus has migrated well into the gradient, whereas all fusion activity has remained on top. This finding indicates that virus particles are not necessary for CE-induced exogenous fusion, thereby suggesting that this phenomenon is fundamentally distinct from paramyxovirus-induced exogenous fusion.

Effect on exogenous fusion of antisera directed against HSV envelope or capsid proteins. To determine whether viral structural components present in the CE were responsible for exogenous fusion, studies were done using immune sera prepared against either solubilized HSV envelope or capsid proteins (see Materials and Methods). Antiserum directed against the smaller envelope component of Newcastle disease virus has been reported to inhibit exogenous fusion induced by that virus (21). In contrast, the results of this study show that antisera directed specifically against either solubilized HSV envelope or capsid proteins had no effect on exogenous fusion activity even though, at least in the case of the anti-envelope protein antiserum, viral infectivity was diminished by nearly 90% (Table 2). This finding suggests that HSV-induced exogenous fusion is mediated by one or more molecules not found as structural components of the virion and is in agreement with results obtained by Ludwig et al. (15).

Biochemical characterization of the HSV fusion factor(s). Additional studies using enzymes and lectins were undertaken to characterize biochemically the macromolecule(s) responsible for HSV-induced exogenous fusion. To assess the effects of various enzymes on exogenous fusion activity, the enzymes were

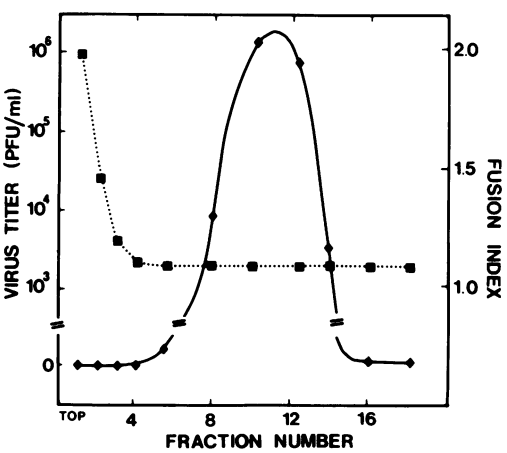

FIG. 4. *Velocity sedimentation centrifugation of fusion-active CE. Aliquots of fusion-active CE (2 ml) were gently layered on top of continuous 20 to 60% sucrose gradients (wt/vol) and centrifuged at 80,000 × g for 2 h. Fractions (2 ml) were collected dropwise from the bottom of each tube and assayed for exogenous fusion activity (■) and viral infectivity (◆).*

first covalently linked to glass beads and then this complex was interacted with fusion-active CE as described in Materials and Methods. Trypsin and two glycosidases, α-L-fucosidase and β-galactosidase, each significantly diminished exogenous fusion activity, whereas several other enzymes were found to have no effect (Table 3). These findings suggest that a protein component in the CE as well as certain sugar residues are involved in the biological activity of the HSV fusion factor(s).

The involvement of specific sugar residues in HSV-induced fusion is further supported by studies using lectins, which are summarized in Table 4. Fucose-binding protein, concanavalin A, and wheat germ agglutinin (used at the indicated concentrations) were each found to exert some inhibitory effect on exogenous fusion. On the other hand, it is seen that identical pretreatment of the indicator monolayers with these lectins had no effect on exogenous fusion, thereby discounting the possibility that the binding of lectin to the indicator cell surface is alone sufficient to inhibit fusion.

TABLE 2. *Effect on exogenous fusion and viral infectivity of antisera directed against HSV envelope or capsid proteins*[a]

Sample	Virus titer (PFU/ml)	% HSV CE virus titer	Fusion index	% HSV CE fusion index[b]
Uninfected control CE			1.08	
HSV CE	4.46×10^7	100	1.88	100
HSV CE plus anti-envelope antiserum	0.91×10^7	13.6	1.93	105.6
HSV CE plus anticapsid antiserum	4.35×10^7	97.5	1.89	96.0

[a] All samples were incubated for 1 h at 37 C before being assayed for residual viral infectivity and fusion activity as described in Materials and Methods.

[b] FI, Fusion index. [(FI after treatment − control CE FI)/(HSV CE FI − control CE FI)] × 100.

TABLE 3. *Effect of enzyme interaction on HSV-induced exogenous fusion*[a]

Enzyme interacted with CE	Fusion index	% HSV CE fusion index[b]
HSV CE	1.91	100
Uninfected control CE	1.10	0
Trypsin (100 μg/ml)	1.14	4.4
α-Fucosidase[c] (2 μg/ml)	1.36	32.1
β-Galactosidase[c] (50 μg/ml)	1.34	29.6
Pyruvate kinase[c] (50 μg/ml)	1.82	88.8
Glucose oxidase[c] (10 μg/ml)	1.96	106.1
DNase (10 μg/ml)	1.83	90.1
RNase (10 μg/ml)	1.86	93.8

[a] Fusion-active HSV CE was incubated for 1 h at 37 C with specified enzyme-glass bead complex before being assayed for residual fusion activity.

[b] For explanation, see footnote *b*, Table 2.

[c] Cross-linked to glass beads, see text (with 77% of protein cross-linked).

DISCUSSION

In this study, we report a model system for HSV-induced exogenous fusion, which can be utilized to elucidate the biological parameters of this phenomenon as well as the biochemical properties of the macromolecule(s) responsible for it. While fulfilling many of the criteria for virion-induced exogenous fusion (e.g., independence from indicator cell macromolecular synthesis), several of the properties of this system are distinct. Particularly, unlike paramyxovirus-induced exogenous fusion, the phenomenon of HSV-induced exogenous fusion has

TABLE 4. *Effect of lectins on HSV-induced exogenous fusion*

Sample	Fusion index	% HSV CE fusion index[a]
Lectin interaction with CE[b]		
HSV CE	1.84	100
Uninfected control CE	1.10	0
Fucose-binding protein (100 μg/ml)	1.22	16.2
Concanavalin A (100 μg/ml)	1.39	39.1
Wheat germ agglutinin (10 μg/ml)	1.68	78.3
Pretreatment of indicator cells with lectin[c]		
HSV CE	1.85	100
Uninfected control CE	1.10	0
Fucose-binding protein (100 μg/ml)	1.89	105.3
Concanavalin A (100 μg/ml)	1.80	93.3
Wheat germ agglutinin (10 μg/ml)	1.93	110.6

[a] For explanation, see footnote *b*, Table 2.

[b] Fusion-active HSV CE was incubated with a lectin at the indicated concentration for 1 h at 37 C before being assayed for residual fusion activity.

[c] Indicator monolayers were interacted with a lectin at the indicated concentrations for 1 h at 37 C. The monolayers were then washed thoroughly with KIR before being overlaid with fusion-active HSV CE.

no requirement for virus particles (see Fig. 4) and possesses a less specific divalent cation requirement than that reported for exogenous fusion induced by Sendai virions (18) (see Fig. 3). Also, HSV-induced exogenous fusion is not inhibited by neutralizing antiserum directed against HSV envelope components, contrary to findings reported for Newcastle disease virus (21), thereby suggesting that HSV-induced exogenous fusion is mediated by a factor(s) that is not a structural component of the HSV virion. Electron micrographs of the CE reveal it to contain vesicular structures that may be derived from the plasma membrane (D. B. Levitan and H. A. Blough, submitted for publication).

Unlike paramyxovirions, herpesvirions have not been demonstrated to fuse cells exogenously. Differences in membrane-mediated biological properties may be explained, in part, by different sites of envelopment of these two virus groups. The paramyxovirus nucleocapsid acquires its envelope by budding through the host cell plasma membrane (1, 2), whereas the herpesvirus envelope is acquired at the inner nuclear membrane of the infected host cell (22). It may be that HSV-specified macromolecules are incorporated selectively into different cellular membranes, or that the viral-coded factor(s) responsible for herpesvirus fusion is transported from the sites of synthesis and incorporated preferentially into host cell plasma membrane while being excluded from or incorporated to a lesser extent into nuclear membrane and, hence, viral envelopes. Alternatively, the viral-specified factor(s) responsible for fusion may be incorporated into the host cell nuclear membrane in an inactive form, namely, not having undergone a post-translational modification which could be essential for biological activity (11, 20).

Experiments using glycosidases and lectins as molecular probes suggest that the HSV fusion factor(s) is a fucose-containing glycoprotein (Tables 3 and 4). Inhibitor studies reported from this laboratory using 2-deoxy-D-glucose have previously indicated that the fusion factors for HSV as well as for Newcastle disease virus are glycoproteins (9). Other workers have reported successful isolation of glycoprotein fusion factors from at least two strains of purified paramyxovirions, Sendai virus (12, 20) and measles virus (10), both of which are able to induce exogenous fusion. The methods used in those studies to isolate a fusion factor from the virion envelope would not, however, be applicable to isolation of the HSV fusion factor, in that our findings suggest strongly that the glycoprotein responsible for HSV-induced exogenous fusion is not a structural component of that virus. We are currently investigating whether the biologically active fusion factor is indeed viral specified or, alternatively, a host cell macromolecule that has undergone a viral-dependent modification. The system we report provides, though, an alternative investigative approach for examining the biological parameters and biochemical properties of viral fusion factors.

ACKNOWLEDGMENTS

This study was supported by grants from the U.S. Public Health Service through the National Cancer Institute (1 RO1 CA15370) and the Damon Runyon-Walter Winchell Memorial Fund for Cancer Research (DRG-1254), and by a contract from U.S. Army Medical Research and Development Command (DADA 17-72C-2147). D.B.L. was the recipient of a summer student fellowship from Fight for Sight, Inc., New York, N.Y. (funded by the Burroughs-Wellcome Corp., Ltd.) and a Public Health Service predoctoral traineeship from the National Eye Institute (5TO1 EY 0049-08).

LITERATURE CITED

1. **Berkaloff, A.** 1963. Étude au microscope électronique de la morphogenese de la particule du virus Sendai. J. Microsc. (Paris) 2:633-640.
2. **Blough, H. A.** 1964. Role of the surface state in the development of myxoviruses, p. 120-151. *In* G. E. W. Wolstenholme and J. Knight (ed.), Ciba Foundation Symposium: cellular biology of myxovirus infections. J. & A. Churchill, Ltd., London.
3. **Bratt, M. A., and W. R. Gallaher.** 1970. Comparison of fusion from within and fusion from without by Newcastle disease virus. In Vitro 6:3-14.
4. **Cohen, G. H., R. K. Vaughan, and W. C. Lawrence.** 1971. Deoxyribonucleic acid synthesis in synchronized mammalian KB cells infected with herpes simplex virus. J. Virol. 7:783-791.
5. **Cohen, G. H., and W. C. Wilcox.** 1966. Soluble antigens of vaccinia-infected mammalian cells. I. Separation of virus-induced soluble antigens into two classes on the basis of physical characteristics. J. Bacteriol. 92:676-686.
6. **Dulbecco, R., and M. Vogt.** 1954. Plaque formation and isolation of pure lines of poliomyelitis viruses. J. Exp. Med. 99:167-182.
7. **Ejercito, P. M., E. D. Kieff, and B. Roizman.** 1968. Characterization of herpes simplex virus strains differing in their effects on the social behavior of infected cells. J. Gen. Virol. 2:357-364.
8. **Flanagan, J. F.** 1967. Virus-specific ribonucleic acid synthesis in KB cells infected with herpes simplex virus. J. Virol. 6:583-590.
9. **Gallaher, W. R., D. B. Levitan, and H. A. Blough.** 1973. Effect of 2-deoxy-D-glucose on cell fusion induced by Newcastle disease and herpes simplex viruses. Virology 55:193-201.
10. **Hall, W. W., and S. J. Martin.** 1974. The biochemical and biological characteristics of the surface components of measles virus. J. Gen. Virol. 22:363-374.
11. **Homma, M., and S. Tamagawa.** 1973. Restoration of the fusion activity of L cell-borne Sendai virus by trypsin. J. Gen. Virol. 19:423-426.
12. **Hosaka, Y., and Y. K. Shimizu.** 1972. Artificial assembly of envelope particles of HVJ (Sendai virus). I. Assembly of hemolytic and fusion factors from envelopes solubilized by Nonidet P40. Virology 49:627-639.
13. **Keller, J. M., P. G. Spear, and B. Roizman.** 1970.

Proteins specified by herpes simplex virus. III. Viruses differing in their effects on the social behavior of infected cells specify different membrane glycoproteins. Proc. Natl. Acad. Sci. U.S.A. 65:865-871.
14. **Kohn, A.** 1965. Polykaryocytosis induced by Newcastle disease virus in monolayers of animal cells. Virology 26:228-245.
15. **Ludwig, H., H. Becht, and R. Rott.** 1974. Inhibition of herpes-virus-induced cell fusion by concanavalin A, antisera, and 2-deoxy-D-glucose. J. Virol. 14:307-314.
16. **Nii, S., and J. Kamahora.** 1961. Cytopathic changes induced by herpes simplex virus. Biken J. 4:255-270.
17. **Okada, Y.** 1972. Fusion of cells by HVJ (Sendai virus), p. 371-382. *In* C. Fred Fox (ed.), Membrane research, first ICN-UCLA symposium on molecular biology. Academic Press Inc., London.
18. **Okada, Y., and F. Murayama.** 1966. Requirement of calcium ions for the cell fusion reaction of animal cells by HVJ. Exp. Cell Res. 44:527-551.
19. **Roizman, B.** 1962. Polykaryocytosis. Cold Spring Harbor Symp. Quant. Biol. 27:327-342.
20. **Scheid, A., and P. W. Choppin.** 1974. Identification of biological activities of paramyxovirus glycoproteins. Activation of cell fusion, hemolysis, and infectivity by proteolytic cleavage of an inactive precursor protein of Sendai virus. Virology 57:475-490.
21. **Seto, J. T., H. Becht, and R. Rott.** 1974. Effect of specific antibodies on biological functions of the envelope components of Newcastle disease virus. Virology 61:354-360.
22. **Siegert, R. S., and D. Falke.** 1966. Electron microscopic investigation of the development of *Herpes hominis* virus in culture cells. Arch. Gesamte Virusforsch. 19:230-240.
23. **Spear, P. G.** 1976. Membrane proteins specified by herpes simplex viruses. I. Identification of four glycoprotein precursors and their products in type 1-infected cells. J. Virol. 17:991-1008.
24. **Treadwell, P. G., and J. R. Ross.** 1962. Effects of human adult and bovine fetal serum on colonial propagation of rabbit cells. J. Natl. Cancer Inst. 28:679-689.
25. **Watson, D. H., W. I. H. Shedden, A. Elliot, T. Tetsuka, P. Wildy, D. Bourgaux-Ramoisy, and E. Gold.** 1966. Virus specific antigens in mammalian cells infected with herpes simplex virus. Immunology 11:399-408.
26. **Wheeler, C. E.** 1964. Biologic comparison of syncytial and a giant cell-forming strain of herpes simplex. J. Immunol. 93:749-756.