Type-Common CP-1 Antigen of Herpes Simplex Virus Is Associated with a 59,000-Molecular-Weight Envelope Glycoprotein

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Received for publication 1 March 1978

The CP-1 antigen of herpes simplex virus type 1 (HSV-1) is a glycoprotein found in the soluble portion of infected cells, in detergent extracts of infected cell membranes, and in the envelope of purified virus. Antisera were prepared against a further purified form of CP-1 prepared from HSV soluble antigen mix; a glycoprotein, gp52, isolated from detergent-treated infected cells; and detergent extracts of purified virus. Each of the antisera reacted with CP-1 to give a single immunoprecipitin band of identity, and each antiserum neutralized the infectivity of HSV-1 and HSV-2. Our results suggested that the type-common determinants involved in the stimulation of neutralizing antibody resided on a 52,000-molecular-weight (52K) glycoprotein. The envelope of HSV contains several glycoproteins: one component at 59K and a complex of two or three components at 130K, none of which corresponds in molecular weight to gp52. Using the antisera as immunological probes, we performed pulse-chase experiments with [³⁵S]methionine-labeled HSV-1-infected cells and followed the disposition of the glycoproteins during the infectious cycle. Each antiserum immunoprecipitated a ³⁵S-labeled 52K protein from lysates of cells pulse-labeled at 5 h after infection. By 10 h, the label was chased into a 59K protein also precipitable by each of the three antisera. The results suggest that gp52 is a precursor of gp59 and that the latter corresponds in molecular weight to one of the major glycoproteins of the virion envelope.

Herpes simplex virus (HSV) glycoproteins are important structural components of the virion envelope and have been implicated in a number of virus-induced biological functions in mammalian cells (10, 14, 22, 23, 29). Previously, we reported the isolation of an HSV-specific fraction from the soluble extract of HSV type 1 (HSV-1)-infected KB or BHK cells which contained an antigen, designated CP-1, having the properties of a glycoprotein (2, 3). Antiserum produced against CP-1 neutralized the infectivity of both HSV-1 and HSV-2. This antiserum reacted against extracts of HSV-infected cells as well as the plasma membrane of productively infected cells and HSV-1-transformed cells (3, 18, 21). The CP-1 antigen also shared immunological properties with the HSV structural band II antigen (3, 20). We suggested that the CP-1 fraction contained a biologically active HSV type-common structural glycoprotein(s) located in the virion envelope as well as in the plasma

membrane and cytoplasm of infected cells.

The purpose of this paper is to extend our previous studies and (i) further characterize CP-1 as it exists in infected cell extracts and (ii) determine whether CP-1 is a structural component of the virion. Using monoprecipitin anti-CP-1 sera as well as antiserum to detergent extracts of purified virions, we are able to demonstrate that CP-1 activity in HSV-1-infected cell extracts is associated with a 52,000-molecular-weight (52K) glycoprotein (gp52) and that this same antigenic activity corresponds to a 59K glycoprotein (gp59) in the virion envelope. Furthermore, we present evidence based on pulse-chase experiments that gp52 is a precursor of the type-common gp59 of the HSV envelope.

MATERIALS AND METHODS

Cell cultures. Conditions for the growth and maintenance of KB cells and baby hamster kidney cells have been previously described (3).

Virus preparation and titration. The procedures used for the preparation of virus stocks of HSV-1 (strain HF) and HSV-2 (strain Savage; for an evaluation of this strain, see reference 6) as well as the plaque assay were described previously (2, 3). For infection,

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unless otherwise noted, an input multiplicity of 10 PFU of HSV per cell was employed.

Labeling of the cells. Isotopically labeled soluble antigen mix (SAM) was obtained from infected KB cells by incubating the culture for 15 h in medium containing [U - 14 C]glucosamine (0.5 μ Ci/ml; specific activity = 250 mCi/mmol) or [35 S]methionine (1 μ Ci/ml; specific activity > 200 Ci/mmol). The radioactive label was usually added 5 h after infection. Radioactive isotopes were purchased from New England Nuclear Corp.

Immunological procedures. Antisera directed against viral antigens were prepared as previously described (3). A guide to the preparation of antisera used in this study is indicated in the footnotes of Table 2. Complement fixation tests were performed as described by Sever (24). Gel diffusion and immunoelectrophoresis were performed as described previously (3). Serum neutralization titers were determined by the plaque reduction technique as previously described (18). The greatest dilution of serum causing a 50% reduction in titer as compared with preimmune serum was selected as the neutralizing titer. Serum-blocking activity was performed as described previously (3).

Purification of CP-1 from SAM. The scheme for purification of CP-1 antigen from the high-speed supernatant fluid of HSV-1-infected cells is outlined below. All procedures were carried out at 4°C. (Step 1) Infected cells were harvested, washed, and subjected to sonic treatment 20 h after infection as previously described (3). The sonic extracts were centrifuged at $6,000 \times g$ for 30 min to remove cell debris. The supernatant was centrifuged at $250,000 \times g$ for 2 h, and the high-speed supernatant was defatted by passage through a sintered-glass filter, then dialyzed against several changes of phosphate-buffered saline (PBS). The pellet was saved for detergent extraction of lipid-soluble components. (Step 2) The supernatant fluid was brought to 40% saturation at 4°C by addition of 23.1 g of solid ammonium sulfate per 100 ml of solution, stirred for 2 h, and centrifuged at $13,000 \times g$ for 30 min. The 40% precipitate was dissolved in cold 1 mM sodium phosphate (PB), pH 7.2, containing 4 mM NaCl and dialyzed exhaustively against the same buffer. The supernatant fraction was adjusted to 75% saturation at 4°C by addition of 22.6 g of ammonium sulfate per 100 ml of solution, dissolved in PB, and dialyzed. In each of the chromatographic procedures (steps 3 through 6) presented in Table 1, column eluates were screened for antigenic activity by gel diffusion and complement fixation employing anti-crude CP-1 serum (3). Fractions containing the greatest antigenic activity were pooled and subjected to the next procedure. (Step 3) The dialyzed extract was applied to a calcium phosphate column (2.54 by 33 cm) prepared and eluted as described previously (3). The fraction eluting between 1 mM and 40 mM phosphate, which contained most of the CP-1 activity, was pooled, concentrated (Amicon, PM10), and dialyzed against 0.01 M phosphate, pH 6.0. (Step 4) The calcium phosphate fraction was applied to a CM-Sephadex C25 (Pharmacia Fine Chemicals, Inc.) column (2.54 by 28 cm) with 0.01 M PB, pH 6.0, at a flow rate of 40 ml/h. The CP-1 antigen was removed in the

wash from this column. (Step 5) The eluate from CM-Sephadex was dialyzed against 0.05 M Tris buffer, pH 7.5, concentrated, and applied to a DEAE-cellulose 23 column (Whatman) in the same buffer. A gradient of NaCl ranging from 0.05 to 0.3 M was applied, and the CP-1 antigen was eluted at a NaCl concentration of 0.14 M. This peak was concentrated and dialyzed against PB. (Step 6) The concentrated, dialyzed extract from DEAE was applied to a second column of calcium phosphate (1 by 9 cm) and eluted with stepwise increases in phosphate concentration (pH 7.2). The CP-1 antigen eluted at 1 and 6 mM phosphate. In this paper we will designate the CP-1 antigenic fraction as originally isolated (3) as crude CP-1 and the antiserum to that fraction as anti-crude CP-1. The antiserum made against the more purified form of CP-1 will be referred to as anti-CP-1. Protein was determined by the method of Lowry et al. (13).

Preparation of detergent extracts of infected cell membranes. The pellet from the high-speed extract of sonically disrupted infected KB cells (step 1 above) was employed as the starting material for detergent extraction of viral antigens. The pellet was resuspended in PBS by sonic treatment and treated with 10 μ g each of DNase and RNase per ml for 1 h at 37°C. This preparation, which contained virions, nucleocapsids, cell membranes, etc. and was presumably devoid of cytoplasmic sap and soluble antigens, was centrifuged at $200,000 \times g$ for 2 h and resuspended in PBS. We were unable to detect diffusible antigens in this preparation. Nonidet P-40 (NP-40) and sodium deoxycholate were each added at a final concentration of 0.5%. The mixture was incubated at 37°C for 1 h and centrifuged at $200,000 \times g$ for 2 h. The supernatant fluid was dialyzed exhaustively against 1 mM PB, pH 6.8, containing 0.1% NP-40 and submitted to calcium phosphate chromatography as described above for CP-1.

Virus purification. HSV-1 labeled with [35 S]methionine or [14 C]glucosamine was purified from the cytoplasmic fraction of infected KB cells by employing methods developed by Spear and Roizman (26) or from the extracellular fluid of HSV-infected cells by the technique of Lawrence (12) and Vernon et al. (27). Purification procedures were monitored by electron microscopy. The polypeptide composition of virus purified by either method was the same. To solubilize the HSV envelope, purified virions were suspended in 0.01 M Tris buffer (pH 7.5) containing 0.15 M NaCl and 0.5% NP-40 and incubated for 45 min at 37°C. Nucleocapsids were removed by centrifugation at $100,000 \times g$ for 1 h.

Pulse-chase experiments. Pulse-chase experiments were performed by essentially the same method as that described by Vogt et al. (28). Briefly, monolayer cultures of KB cells in 60-mm plates were infected with 40 PFU of HSV-1 per cell. At 5 h after infection, the medium was decanted, and the cells were washed once with warmed Hanks salts and incubated at 37°C for 15 min in 1.5 ml of Hanks salts containing 200 μ Ci of [35 S]methionine. For pulse experiments, the monolayers were washed twice with 10 ml of iced saline and lysed by addition of 1 ml of cold lysing buffer (0.05 M NaCl-0.5% sodium deoxycholate-0.5% NP-40 in 0.02 M Tris, pH 7.5). Lysed cells were scraped into 3-ml

conical centrifuge tubes, blended in a Vortex mixer for 30 s, and centrifuged at $2,000 \times g$ to remove nuclei and cell debris and then at $100,000 \times g$ for 1 h. In chase experiments, the salt solution containing the label was decanted, and the monolayer was washed three times with complete medium and incubated in 10 ml of the same medium for an additional 5 h. At the end of that time, cells were washed and treated with lysing buffer in the same way as for the pulse experiments.

For immune precipitation, 0.1 ml of antiserum was added to 50 μ l of the supernatant fluid, and the mixture was incubated at 37°C for 1 h, followed by incubation at 4°C for 4 to 6 days. Immune precipitates were collected by centrifugation, washed three times with lysing buffer, then once with distilled water, and dissolved in electrophoresis sample buffer described below.

Electrophoresis on SDS-PAGE. For sodium dodecyl sulfate-polyacrylamide gel (SDS-PAGE) electrophoresis, portions of SDS-solubilized infected cell extracts or immune precipitates were subjected to electrophoresis in slabs of acrylamide cross-linked with *N,N*-methylenebisacrylamide (11). Separation gels contained 10% acrylamide monomer and 0.26% *N,N*-methylenebisacrylamide by weight. Stacker gels were 5% acrylamide cross-linked with 0.13% bisacrylamide. The sample buffer was 0.1 M Tris (pH 6.8)–1% 2-mercaptoethanol–5% SDS–0.002% bromophenol blue. Samples were heated in 50 μ l of this buffer for 2 min at 100°C before application to the gel. The gels were run at 22.5 mA for 3 to 4 h. After electrophoresis the gels were fixed and stained with 0.2% Coomassie brilliant blue in 50% methanol–7% acetic acid, then destained in the same solution minus the Coomassie brilliant blue. For autoradiography, the gels were dried on filter paper and placed in contact with Kodak XRP-54 film. For fluorography the gels were impregnated with 2,5-diphenyloxazole (1), placed in contact with Kodak XRP-54 film and stored at –70°C. The following proteins were run to aid in molecular weight determinations: *Escherichia coli* β -galactosidase (130,000 daltons), human transferrin (95,000 daltons), bovine serum albumin (67,000 daltons), beef liver catalase (60,000 daltons), bovine immunoglobulin G heavy chain (53,000 daltons), pig heart fumarase (49,000 daltons), ovalbumin (45,000 daltons), yeast glyceraldehyde 3-phosphate dehydrogenase (36,000 daltons), bovine pancreas chymotrypsinogen (25,000 daltons), bovine immunoglobulin G light chain (23,000 daltons), and bovine hemoglobin (15,000 daltons). All proteins except bovine immunoglobulin G were obtained from Sigma Chemical Co., St. Louis, Mo. Bovine immunoglobulin G was purchased from Miles Laboratories, Elkhart, Ind.

RESULTS

Glycoprotein synthesis in KB cells infected with HSV. Figure 1 shows the radioactive profile of [14 C]glucosamine-labeled molecules synthesized in HSV-infected (Fig. 1A) and mock-infected KB cells (Fig. 1B). A number of virus-induced glycoproteins appeared after infection which were distributed on SDS-PAGE into two broad peaks centering around 130K and

59K. These findings are consistent with those reported by a number of investigators (16, 20, 26). The following experiments were designed to locate and characterize the glycoprotein(s) associated with CP-1 activity.

Purification of the CP-1 antigen from HSV-1-infected cell extracts. A summary of the procedure employed for the purification of [14 C]glucosamine-labeled CP-1 antigen is presented in Table 1. After each step, eluates were screened for antigenic activity employing the monoprecipitin anti-crude CP-1 serum (3). The six-step procedure (see above) resulted in a 146-fold increase in the specific activity of the glucosamine label and a 68-fold increase in antigenic activity as measured by complement fixation. This further purified form of CP-1 was used to prepare antiserum (designated anti-CP-1).

Characterization of the CP-1 antigen. (i) Gel diffusion analysis. A single common immunoprecipitin band was obtained when CP-1

FIG. 1. Absorbance tracings from autoradiograms of electrophoretically separated (SDS-PAGE) [14 C]-glucosamine-labeled polypeptides derived from (A) HSV-1-infected cell extracts labeled with [14 C]glucosamine at 5 to 18 h after infection and (B) uninfected cell extracts labeled with [14 C]glucosamine for 15 h. Absorbance scales for these tracings are directly comparable.

was reacted with the following antisera: anti-crude CP-1, anti-CP-1, anti-SAM, and anti-HSV-ENV (to be discussed below). The same results were obtained with CP-1 labeled with [14 C]glucosamine (Fig. 2A) or with [35 S]methionine (data not shown). These results indicated that CP-1 contained a glycoprotein, in agreement with previous results for crude CP-1 (3). Immuno-electrophoretic analysis of the [14 C]glucosamine-labeled CP-1 antigen (Fig. 2B) indicated that all of the radioactive label was associated with a single immunoprecipitin band. The spurring of the arc suggested that the antigen was not homogeneous in composition.

(ii) **Biological activity.** The biological activity of CP-1 was preserved after purification as judged by two criteria: (i) ability to stimulate production of neutralizing antibody (Table 2) and (ii) capacity to block serum neutralizing

TABLE 2. Serum neutralization of HSV infectivity

Antiserum prepared against:	Neutralization titer ^a	
	HSV-1	HSV-2
SAM	600	128
Crude CP-1 ^b	512	128
CP-1 ^c	192	128
gp52 ^d	128	128
HSV-ENV ^e	512	128

^a Greatest dilution of serum which leads to a 50% reduction in infectivity.

^b SAM was chromatographed on calcium phosphate and eluted at 40 mM phosphate (3). Serum was prepared against the dialyzed, concentrated fraction.

^c Serum prepared against further purified CP-1 (step 6, Table 1).

^d Serum prepared against 52K glycoprotein isolated from a detergent extract of infected cells (Fig. 4B).

^e Serum prepared against detergent extract of purified virus, i.e., envelope (Fig. 7B and C).

TABLE 1. Purification of CP-1 from SAM^a

Procedure	Fraction	Total protein (mg)	Sp act	
			[14 C]glucosamine (cpm/mg of protein)	CF titer ^b (units/mg of protein)
1. Centrifugation	SAM	1,120	1,000	480
2. Ammonium sulfate	40-75%	408	810	560
3. Calcium phosphate I	10 mM PO ₄	59	3,900	1,425
4. CM-Sephadex	Wash	35	5,050	3,300
5. DEAE-cellulose	0.14 M NaCl	7.8	11,600	6,150
6. Calcium phosphate	1 mM PO ₄ + 6 mM PO ₄	0.18	146,000	32,700

^a SAM was prepared from 10¹⁰ infected KB cells.

^b CF titer, Complement fixation titer of anti-crude CP-1 serum (3).

FIG. 2. (A) Autoradiograph derived from the gel diffusion analysis of [14 C]glucosamine-labeled CP-1. Center well, purified CP-1 antigen. Peripheral wells: (1) anti-crude CP-1 serum (reference 3); (2) anti-CP-1 serum; (3) anti-SAM serum; (4) anti-HSV-ENV serum. The agar was washed extensively with saline, dried, and exposed to Kodak XRP-54 film. (B) Immuno-electrophoretic analysis of [14 C]glucosamine-labeled CP-1. Trough (a), anti-SAM. Center well (b), purified CP-1 antigen. The agar was dried and exposed to Kodak XRP-54 film. See Table 2 for guide to antisera.

activity (data not shown). Table 2 shows also that anti-CP-1 serum neutralized both HSV-1 and HSV-2, indicating that CP-1 contains a type-common structural antigen.

(iii) **SDS-PAGE analysis.** Figure 3 shows that CP-1 was resolved into two major [14 C]-glucosamine-labeled bands with apparent molecular weights of 52K and 47K and representing over 95% of the total radioactivity. Minor radioactivity bands at 130K, 43K, and 28K were detected after long-term exposure of the gel. The results suggest several possibilities: (i) CP-1 was still contaminated with cellular components, (ii) CP-1 was part of a membrane complex containing several other unrelated glycoproteins, or (iii) the glycoproteins represented antigenically related but electrophoretically distinct glycoproteins in the cell (8, 25). Furthermore, it is possible that some of these bands represented degradation products produced during purification. However, degradative changes extensive enough to lead to a total loss of biological function were unlikely because CP-1 was able to stimulate production of neutralizing antibody.

The more interesting possibility is based on the suggestion of Spear (25) and Honess and Roizman (8) that at least some of the glycoproteins present in infected cell lysates are underglycosylated precursors of virion glycoproteins. It seemed possible, therefore, that the population of proteins and glycoproteins present in CP-1 included some precursor forms of the mature type-common glycoprotein found in virion membrane. For this reason we decided to examine those glycoproteins extractable by detergent (i) from membranes of infected cells and (ii) from purified virions to determine whether the CP-1 antigen was present.

FIG. 3. Absorbance tracing (graph) of autoradiogram (insert) of electrophoretically separated (SDS-PAGE) [14 C]glucosamine-labeled CP-1.

Glycoproteins extracted from detergent-solubilized HSV-1-infected cell pellets. HSV-infected cells were subjected to sonic treatment and to high-speed centrifugation (see step 1 above). The pellet was resuspended in buffer, subjected to sonic treatment again, and recentrifuged. This pellet was resuspended, subjected to sonic treatment, and assayed by gel diffusion for soluble antigens by employing anti-HSV-SAM and anti-CP-1 sera. We were unable to detect CP-1 or any other diffusible HSV antigens in this material. The pellet was treated with lysing buffer (above) and centrifuged at high speed to pellet detergent-insoluble material. Figure 4 shows the radioactive profile obtained when the supernatant from detergent-treated [14 C]glucosamine-labeled infected cells was chromatographed on a calcium phosphate column. The eluates were screened for CP-1 activity and pooled into three fractions as indicated in Fig. 4. Fractions I and II reacted strongly with anti-CP-1 serum (by gel diffusion), and fraction III reacted weakly. The fractions were analyzed by SDS-PAGE electrophoresis (Fig. 5). The total extract before chromatography on calcium phosphate (Fig. 5A) contained two major glucosamine-labeled bands (gp130 and gp52) and two minor bands (gp59 and gp110). Fraction I (Fig. 5B) appeared to contain only gp52. Fraction II (Fig. 5C) contained gp52 and a small amount of gp130 which is not apparent in Fig. 5C. Fraction III contained gp130 as well as small amounts of gp110 and gp59 but no detectable gp52. Although fraction I contained only gp52, the Coomassie brilliant blue-stained gel showed additional bands at 23K and 53 to 55K. These additional bands probably correspond to serum contaminants.

Antiserum prepared against fraction I (Fig. 5B, called anti-gp52) was reacted with the HSV-soluble antigen mix and gave a single immunoprecipitin band which showed identity with anti-CP-1 serum (Fig. 6). Table 2 shows that anti-gp52 serum neutralized both HSV-1 and HSV-2. We conclude that the detergent-solubilized portion of infected cells contains a 52K glycoprotein, gp52, which appears to be antigenically indistinguishable from CP-1 purified from the cytoplasm. Having found CP-1 antigen in both soluble and detergent-solubilized fractions of infected cells, we next wanted to see whether this antigen could also be found in the envelope of purified virus.

Glycoproteins extracted from the lipid envelope of purified HSV virions. (i) **Gel diffusion analysis of the NP-40 extract.** Purified virions labeled with [35 S]methionine or [14 C]glucosamine were extracted with NP-40 (see above), and the extract which contained the CP-1 antigen as well as several other viral pro-

FIG. 4. Calcium phosphate chromatography of detergent extract of infected cells. The cells were labeled from 5 to 18 h postinfection, subjected to sonic treatment, washed free of agar-diffusible CP-1 antigen, and then detergent extracted (as in the text). The material was subjected to calcium phosphate chromatography. For this experiment, 58,000 cpm (optical density at 280 nm = 8.7) were applied to the column in 5 ml of 10^{-3} M potassium phosphate buffer, pH 6.8, containing 0.1 M NaCl. The column size was 1×9 cm, and the flow rate was 40 ml/h. Recovery was 63% by optical density and 51% by radioactivity.

teins was used to prepare antiserum (called anti-HSV-ENV).

(ii) **Biological properties of anti-HSV-ENV.** This serum reacted against CP-1 (Fig. 2A) and gave a band of identity with anti-gp52 and anti-CP-1. This is further evidence that there is an antigen present in infected cells that is also in the virus. The three sera, anti-CP-1, anti-gp52, and anti-HSV-ENV, were able to neutralized HSV-1 and HSV-2 (Table 2).

(iii) **SDS-PAGE analysis.** Figure 7A shows that the [35 S]methionine-labeled virus preparation contained approximately 23 polypeptides. In the NP-40 extract of virions there were glucosamine-labeled bands at 130K and 59K (Fig. 7B). By using [35 S]methionine as the label, additional minor bands were seen at 154K, (major capsid protein) 100K, and 39K (Fig. 7C). These data are essentially in agreement with those of other laboratories (16, 20, 25).

The gel diffusion results (Fig. 2A and 6) indicated that the three antisera prepared in the course of this study (anti-CP-1, anti-gp52, and anti-HSV-ENV) recognized a common antigen, CP-1. Yet, according to the SDS-PAGE analysis (Fig. 7B), none of the glycoproteins extracted from the virus corresponds in molecular weight to the major glycopeptides of CP-1 (Fig. 3) or to gp52 isolated from detergent-solubilized infected-cell pellets (Fig. 5B). Spear (25) showed

that a number of polypeptides in the infected cell are precursors of mature glycoproteins in the virion. Our results could therefore be explained by assuming that gp52 is a precursor to one or more of the mature glycoproteins of the virus. To test this hypothesis, we used our three antisera as immunological probes in pulse-chase experiments to follow the disposition of CP-1 during the infectious cycle.

Evidence that gp52 is precursor to the type-common glycoprotein in HSV virions. Figure 8 shows the labeling pattern of the [35 S]methionine-labeled cytoplasm after a 15-min pulse at 5 h postinfection (track 1) and a 15-min pulse at 5 h after infection followed by a 5-h chase (track 2). Several changes in the polypeptide labeling pattern were observed. Most significant among these changes were alterations in the bands corresponding to the two major structural glycoproteins (arrows). Tracks 3 and 5 show that a major radioactive band at 52K was immunoprecipitated by anti-CP-1 and anti-gp52 from extract labeled during the pulse. Tracks 4 and 6 show that these same two antisera precipitated a more heterogeneous band at approximately 59K after a 5-h chase. These results provide strong evidence that gp52 is a precursor molecule of gp59. Track 7 shows that anti-HSV-ENV also precipitated the 52K protein from pulse-labeled extracts. In addition, one

FIG. 5. Absorbance tracings of autoradiograms of electrophoretically separated (SDS-PAGE) [^{14}C]glucosamine-labeled polypeptides obtained from the calcium phosphate column in Fig. 4. (A) Material before calcium phosphate; (B) fraction I from calcium phosphate; (C) fraction II from calcium phosphate; (D) fraction III from calcium phosphate. Absorbance scales for these tracings are directly comparable.

other major band at approximately 110K was precipitated from the pulse-labeled material by anti-HSV-ENV serum. In the chase (track 8), this same serum precipitated the heterogeneous band at 59K as well as another heterogeneous set of bands at 130K and a small amount of the 110K band seen in the pulse. Studies are now in progress to determine the relationship of the 110K and the several proteins in the 130K region. Long-term exposure of the fluorogram of ^{35}S -labeled immune precipitates revealed the presence of additional labeled bands especially in the 130K region with all of the antisera. These minor bands, however, represented a small fraction of the total label. The results of this experiment indicate that gp52 is a precursor of gp59 found in the envelope of the virion.

DISCUSSION

The results presented in this study demonstrate that the CP-1 antigen of HSV is, at least

FIG. 6. Gel diffusion analysis of anti-gp52 and anti-CP-1. Center well, HSV-SAM. Peripheral wells: (1) anti-gp52, (2) anti-CP-1. The gel was washed extensively with saline, dried, and stained for protein with 1% amido Schwartz in 7% acetic acid. See Table 2 for guide to antisera.

in part, a glycoprotein found in the soluble portion of infected cells (HSV-SAM), in detergent extracts of infected cell membranes, and in the envelope of purified virus. Antisera prepared against: (i) CP-1, purified from HSV-SAM (Table 1), (ii) gp52 isolated from detergent-treated cells (Fig. 5B), and (iii) detergent extracts of purified virus (Fig. 7B and C) are all able to react with purified CP-1. Each of the three antisera is capable of neutralizing the infectivity of HSV-1 and HSV-2 (Table 2). The results employing anti-CP-1 and anti-gp52 sera suggest that type-common determinants involved in the stimulation of neutralizing antibody reside on the 52K glycoprotein component (gp52). From other reports (16, 20, 25) as well as our own experiments (Fig. 7), we know that the envelope of HSV-1 contains several major glycoprotein components, one component with an apparent molecular weight in SDS-PAGE of approximately 59K and a complex of several polypeptides with an average molecular weight of approximately 130K. None of these glycoproteins corresponds in molecular weight to gp52 or to the two major glycopeptides in CP-1. Using each of the three antisera as an immunological probe, we performed pulse-chase experiments with [^{35}S]methionine-labeled HSV-infected cells and followed the disposition of CP-1 during the infectious cycle. Each antiserum immunoprecipitated a ^{35}S -labeled 52K protein from lysates of cells pulse-labeled at 5 h after infection (Fig. 8).

FIG. 7. Absorbance tracings of autoradiograms of electrophoretically separated (SDS-PAGE) molecules obtained from purified virus. (A) Whole virus; (B) [^{14}C]glucosamine-labeled molecules extracted by NP-40 from purified virus. (C) [^{35}S]methionine-labeled molecules extracted by NP-40 from purified virus.

By 10 h after infection, the [^{35}S]methionine label was chased into a 59K protein also precipitated by each of the three antisera. These results suggest that gp52 is a precursor of gp59 and that the latter corresponds in molecular weight to one of the two major glycopeptides of the virion envelope.

Our results confirm earlier studies (8, 25) which showed that the virion glycoproteins of HSV appear to exist in a precursor and mature form in the infected cell. In particular, we propose that gp52 seen in the pulse corresponds to Spear's glycoprotein pD and gp59 seen in the chase (Fig. 8) and in the virion envelope (Fig. 7) corresponds to Spear's D2 (25). The relationship of CP-1 to those reported by other groups is

FIG. 8. Pulse-chase experiment. Fluorogram of a 10% polyacrylamide gel of immune precipitates from lysates of HSV-infected KB cells. The lysates in the odd-numbered tracks were prepared from cells pulse-labeled for 15 min with 200 μCi of [^{35}S]methionine at 5 h after infection. The lysates in the even-numbered tracks were prepared from cells pulse-labeled with [^{35}S]methionine at 5 h after infection and then chased for 5 h in medium containing nonradioactive methionine. Track (1), pulse; track (2), chase; tracks (3) and (4), anti-CP-1 serum; tracks (5) and (6), anti-gp52 serum; tracks (7) and (8), anti-HSV-ENV serum.

shown in Table 3. It is possible that the 52K and 47K molecules that we found to be the major components of CP-1 represent underglycosylated forms of the virion glycoprotein gp59 which accumulate late in infection (25). A similar explanation could account for our finding gp52 in detergent extracts of infected-cell pellets because that material was derived from cells late in infection. We cannot, however, rule out the possibility that the glycoproteins of CP-1 and gp52 isolated from membranes are degradation products that arose during purification. The neutralization data in Table 2 suggest that during the six-step purification, the capacity of the antigen to stimulate HSV-1 type-specific antibody was reduced. These data can be interpreted to indicate that type-specific determinants were either removed or degraded during purification. Thus, for instance, the 47K molecule seen in the CP-1 preparation could represent a degradation product of a type-specific antigen or it could represent another molecular form of CP-1.

Because of the specificity of our anti-CP-1 and anti-gp52 sera, we hypothesize that in the virion

TABLE 3. *Summary of current designations for HSV glycoproteins possibly related to CP-1*

Glycoprotein designation	Mol wt ^a	Biological property	Reference
gp52 (CP-1)	52	Induces type-common neutralizing antibody, precursor glycopeptide	This paper and 3
gp59	59	Structural glycoprotein	
pD	59	Precursor glycopeptide	25
D1		Precursor glycopeptide	
D2	64	Structural glycoprotein	
Vp 19E	53	Structural glycoproteins	7
Vp 18	57		
Vp 17	59		
Ag 8	60 69	Induces type-common antibody	17
8/9	44-59	Induces type-common neutralizing antibody	19
C 8	58		5 and R. Courtney (personal communication)
57	57	Structural glycoproteins	16
51	51		

^a Estimated by migration in SDS-PAGE. Values presented should be multiplied by 1,000.

gp59 is a glycoprotein which stimulates the production of type-common neutralizing activity. This hypothesis suggests that gp59 of HSV participates in the initial stages of the infectious process and that antibody to this structural component interrupts its biological function. Precisely what role gp59 plays in the infectious process remains to be elucidated. There are a number of biological properties associated with the glycoproteins of HSV including attachment, social behavior of infected cells (22), oncogenicity (21), and induction of neutralizing antibody (3, 19). It has been proposed that two of the glycoproteins in the 130 complex are involved in cell fusion (14). It is not clear at this point whether the carbohydrate or peptide portions of the molecule (or both) are important in this or the other biological functions of HSV glycoproteins. All of our antisera were made against molecules that contained some carbohydrate, and it is possible that this carbohydrate might be essential for stimulating neutralizing antibody. However, the carbohydrate that is added during the maturation of gp52 into gp59 appears not to be required for stimulating type-common neutralizing antibody. This idea is based on the observation that anti-HSV-ENV serum contained the same level of type-common neutralizing antibody as anti-CP-1 and anti-gp52 sera (Table 2). The additional type-specific neutralizing activity found in anti-HSV-ENV serum

could have been induced by the extra carbohydrate component of gp59 or by other antigenic components found in NP-40 extracts of purified virus. For example gp130, which is a major component of the virion, has been shown to induce type-specific neutralizing antibody (4, 19). This glycoprotein was a very minor component of CP-1 (Fig. 3) and was virtually absent from fraction I isolated from the nonsoluble portion of infected cells (Fig. 5B). Future studies in our laboratory will be directed at determining the contribution of the carbohydrate and peptide portions of gp59 to antigenicity, induction of neutralizing antibody, and infectivity of the virus.

Although our experiments establish a precursor-product relationship for gp52 and gp59, they leave unanswered the question of whether gp59 and gp130 are related. Even the most specific antisera used in these studies, anti-CP-1 and anti-gp52, precipitated a small but detectable amount of the polypeptides associated with gp130. Our results could be explained in two ways. First, it was quite possible that all of our antisera, including anti-CP-1 and anti-gp52, contained small amounts of antibodies directed against the proteins of gp130, present as minor contaminating components. Alternatively, it is possible that antibodies specifically directed against gp52 had some affinity for the proteins in gp130 due to similarities in either the carbo-

hydrate or peptide portions of the molecule. There is precedent in the literature for differences in carbohydrate radically affecting the mobility of glycoproteins in SDS gels. For example, Marquardt et al. (15) showed that the chief difference between gp45 and gp70 of Rauscher leukemia virus lies in the degree of glycosylation of the two molecules. On the other hand, the data of Spear suggested that the major glycosylated proteins of HSV are derived from antigenically distinct glycoproteins. Her data do not exclude the possibility that some carbohydrate and/or peptides on these proteins are shared. Experiments are now underway in our laboratory to determine the structural relationship between gp52 and gp59 and the relationship of these molecules to the other virion glycoproteins of HSV.

ACKNOWLEDGMENTS

This investigation was supported by Public Health Service grant DE-02623 from the National Institute of Dental Research.

We thank William Lawrence, Paul C. Montgomery, Lewis Pizer, and Wesley Wilcox for their help in the preparation of this manuscript, Heidi Diggelmann for helpful discussions and suggestions, and Steven Vernon for electron microscopy. We also acknowledge the excellent technical assistance of Madeline Cohen.

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