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Identification of virion polypeptides in hamster cells transformed by herpes simplex virus type 1

(surface labeling/immunoprecipitation/polyacrylamide gel electrophoresis)

PHALGUNI GUPTA AND FRED RAPP

Department of Microbiology, The Milton S. Hershey Medical Center and Specialized Cancer Research Center, The Pennsylvania State University, College of Medicine, Hershey, Pa. 17033

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ABSTRACT **Ten polypeptides were detected on the surface of the virion of herpes simplex virus type 1. Of these ten polypeptides, three were detected in hamster cells transformed by herpes simplex type 1.**

Virus-specific antigens have been detected on the plasma membrane of cells productively infected with herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) (1–3). Hamster embryo fibroblast cells transformed by inactivated HSV-1 or HSV-2 also show the continued presence of virus-specific antigens on the cell surfaces but do not release virus particles (4–6). Reed *et al.* (7) have reported the presence of a group of undefined virion-associated antigens, designated CP-1, on the surfaces of hamster cells transformed by inactivated HSV-1. A major difficulty associated with these experiments was that the antigens were detected by immunofluorescence methods, which do not characterize the particular virion polypeptide present in the test cell.

In this report we describe the characterization of herpesvirus polypeptides present in the HSV-1-transformed cells. The search for the virion polypeptides was made logical by the observation that rabbit antiserum to a tumor derived by the inoculation of HSV-1-transformed cells into a syngeneic hamster is capable of strongly neutralizing HSV-1 but not HSV-2. Thus, it was possible to immunoprecipitate the labeled virion surface protein antigens with this rabbit antitumor serum and to identify the antigens by polyacrylamide gel electrophoresis. Using this technique, we found evidence for the presence of three virion-associated proteins in the HSV-1-transformed cells.

MATERIALS AND METHODS

Cells and Virus. HSV-1 (strain Patton) was grown in Vero cells in medium 199 supplemented with 10% fetal calf serum. The purification of virus from infected cells by dextran gradient centrifugation has been described (8). The purified virus was found by electron microscope examination to be 95–97% enveloped and to contain no cellular contamination.

Preparation of Surface-Labeled Virion Envelope. Total HSV-1 purified from 20 roller bottles was surface-labeled with iodine-125 (^{125}I) in the presence of lactoperoxidase and H_2O_2, as described by Philips and Morrison (9). The envelope was separated from the surface-labeled virion by treatment with Nonidet P-40 (NP-40), described elsewhere (8). Briefly, NP-40 was added to the surface-labeled virus to a concentration of 1%; the mixture was incubated in ice for 15 min and then centrifuged at 100,000 × *g* for 90 min at 4°. The supernatant was removed and used as virion surface antigen.

Chromatography on Concanavalin A-Sepharose Column. A 3-ml column of concanavalin A bound to Sepharose 4B (Pharmacia Fine Chemicals) was prepared and equilibrated with buffer I (0.02 M Tris·HCl, pH 7.5, 0.25 M NaCl, and 1 mM each $MgCl_2$, $MnCl_2$, and $CaCl_2$). A 50-μl aliquot of surface-labeled virion envelope antigen (3×10^5 trichloroacetic acid-insoluble cpm) in phosphate-buffered saline containing 1% NP-40 was applied to the column and washed exhaustively with buffer I. The bound material was eluted from the column with 0.1 M methylglucoside in buffer containing 0.02 M Tris·HCl (pH 7.5) and 0.25 M NaCl.

Antisera. The tumor line 14-012-8-1,T-10 (hereafter called tumor 10) was derived by inoculation of 14-012-8-1 (HSV-1-transformed) cells into syngeneic hamsters. The detailed history and propagation of this tumor line are given elsewhere (10). Rabbit antisera against tumor cells were prepared by eight multiple injections of 10^8 tumor cells in Freund's complete adjuvant at three-week intervals. Goat anti-rabbit immunoglobulin G (IgG) was purchased from Miles Laboratories. For serum absorption, small aliquots of antisera against tumor 10 were absorbed with two volumes of packed cell. All absorptions were performed at 37° for 1 hr with continuous mixing.

Immunoprecipitation. The incubation mixture of immunoprecipitation contained 50 μl of TEN buffer (0.02 M Tris·HCl, pH 7.4, 0.1 M NaCl, 0.001 M EDTA) containing 2 mg/ml of bovine serum albumin (fraction V, Sigma Chemical Co.), 25 μl of ^{125}I-labeled virion surface antigen (2×10^5 trichloroacetic acid-insoluble cpm), and increasing amounts of rabbit antiserum against tumor or equivalent amounts of preimmune rabbit serum. The mixture was incubated at 37° for 30 min and then overnight at 4°, after which goat anti-rabbit IgG was added to the incubation mixture. The optimal amount of goat anti-rabbit IgG added was determined in other experiments by titration of different amounts of normal rabbit serum. The immunoprecipitates that formed in 18 hr at 4° were collected by centrifugation at 1700 × *g* for 30 min through 1 M sucrose in TEN. The pellet was washed three times by resuspension in 0.5 ml of TEN containing 0.2% NP-40 and by centrifugation. The radioactivity of the washed pellet was measured directly in a Nuclear Chicago gamma counter or the pellet was analyzed by polyacrylamide gel electrophoresis.

Polyacrylamide Gel Electrophoresis. The immunoprecipitates were solubilized by heating in an electrophoresis buffer containing 0.0625 M Tris·HCl (pH 6.8), 5% sodium dodecyl sulfate ($NaDodSO_4$), and 5% mercaptoethanol for 2 min at 100°, and were then analyzed by a 5–15% gradient polyacrylamide gel electrophoresis in the presence of $NaDodSO_4$ (11). After electrophoresis, the gel was dried and an autoradiogram was prepared using Kodak no-screen x-ray film.

RESULTS

Identification of Polypeptides Present on the Surface of HSV-1 Virions. To identify the proteins present on the surface

Abbreviations: HSV, herpes simplex virus; NP-40, Nonidet P-40; $NaDodSO_4$, sodium dodecyl sulfate.

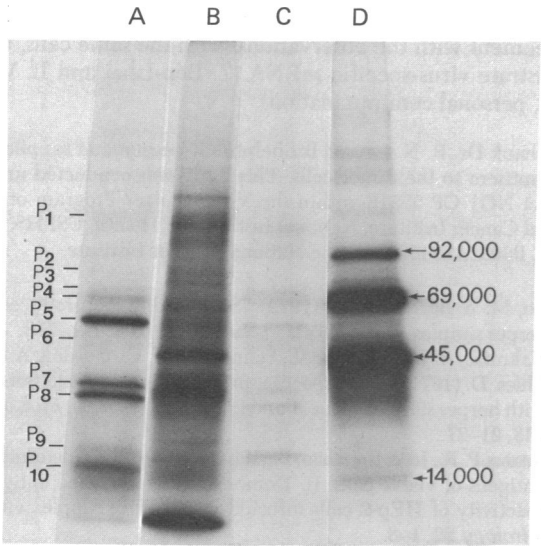

FIG. 1. Polyacrylamide gel electrophoresis of surface-labeled HSV-1 envelope proteins. Proteins were solubilized by heating at 100° in a buffer containing 0.0625 M Tris·HCl (pH 6.8), 5% $NaDodSO_4$, and 5% mercaptoethanol, and were analyzed by polyacrylamide gel electrophoresis on a 5–15% gradient in the presence of $NaDodSO_4$. (Lane A) Surface-labeled HSV-1 envelope; (lane B) HSV-1 was disrupted with 2% $NaDodSO_4$ and then labeled with ^{125}I; (lane C) concanavalin A-bound material of HSV-1 surface-labeled envelope; (lane D) marker proteins, molecular weights indicated by arrows.

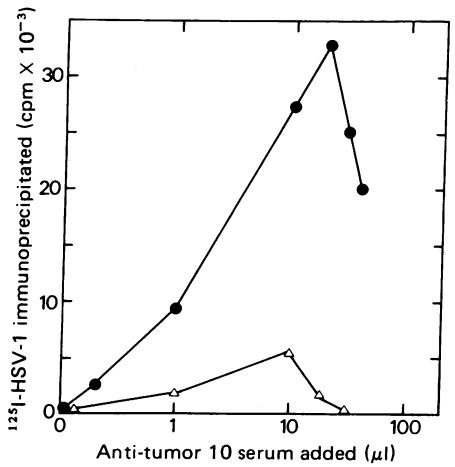

FIG. 2. Immunoprecipitation curve of surface-labeled HSV-1 virion envelope antigen with rabbit anti-tumor 10 (HSV-1) serum (●) and rabbit anti-tumor Cx-90-3B,T-2 (cytomegalovirus) serum (△). Counts per minute in immunoprecipitates were computed after subtraction of the background cpm obtained with an equal volume of preimmune serum.

of HSV-1 virions, we treated enveloped virions with ^{125}I under conditions such that only exposed surface proteins were labeled. Although the virus preparation contained 95–97% enveloped virus, it was necessary to avoid detecting any internal protein that could be labeled by ^{125}I from the few nonenveloped virions (2–3%) present by treating the surface-labeled virus preparation with 1% NP-40 and centrifuging to remove any virion core particles. Analysis of the recovery of the radioactivity after the NP-40 treatment indicated that 90–95% of the labeled virion preparation was associated with the surface proteins (data not included). Fig. 1 shows the analysis by polyacrylamide gel electrophoresis of the surface-labeled envelope of HSV-1 (lane A) together with HSV-1 disrupted with $NaDodSO_4$ prior to labeling with ^{125}I (lane B). As is indicated, the virion surface contains at least six major proteins (molecular weights in parentheses): P_4 (70,000), P_5 (58,000), P_7 (33,000), P_8 (30,000), P_9 (18,000), and P_{10} (15,500); and four minor proteins: P_1 (115,000), P_2 (93,000), P_3 (75,000), and P_6 (48,000). The total number of proteins present in the virion measured by comparable methods is 22 to 24 (8, 12).

To determine whether all of these surface proteins are glycoproteins, we chromatographed the separated labeled envelope proteins of HSV-1 on a concanavalin A-Sepharose column. The material that bound to the column was eluted with 0.1 M methylglucoside and analyzed by polyacrylamide gel electrophoresis (Fig. 1, lane C). As expected, most of these surface proteins are glycoproteins.

Immunoprecipitation with Rabbit Antiserum against Tumor. Surface-labeled HSV-1 envelope antigen was reacted with increasing concentrations of rabbit antiserum against tumor 10. Under optimal conditions, 12–15% of input radioactivity could be reproducibly immunoprecipitated (Fig. 2). The amount of material immunoprecipitated under optimal conditions was solubilized with $NaDodSO_4$-containing buffer and analyzed by polyacrylamide gel electrophoresis as described in *Materials and Methods* (Fig. 3). The immunoprecipitate (lane B) contained four proteins (P_5, P_7, P_8, and a faint band above P_7), indicating the presence of the three major virion proteins in the tumor 10 line. Material derived from HSV-1 and preimmune rabbit sera contained no such polypeptides (lane C). These gel analysis profiles are very reproducible. We do not know the identity of the faint band present just above P_7 (lane B).

When similar procedures for virus purification were applied to mock-infected cells, we could not detect any protein that could be labeled by ^{125}I, indicating an absence of contaminating cellular protein in the purified virus preparation. This was further supported by the fact that preabsorption of antisera against tumor 10 with uninfected Vero cells did not remove immunological reactivity towards surface-labeled HSV-1. To determine the specificity of the immunoprecipitation reaction, we reacted the surface-labeled envelope antigen of HSV-1 with rabbit antisera to a tumor (Cx-90-3B,T-2) produced by inoculation of hamster embryo fibroblast cells transformed by UV-

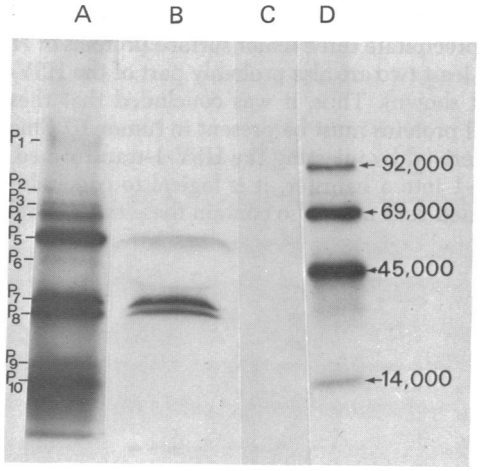

FIG. 3. Polyacrylamide gel electrophoresis of the immunoprecipitate derived from surface-labeled HSV-1 and rabbit anti-tumor 10 serum. (Lane A) Surface-labeled HSV-1 envelope proteins; (lane B) immunoprecipitate derived from surface-labeled HSV-1 envelope proteins and rabbit anti-tumor 10; (lane C) same as lane B, except that anti-tumor 10 serum was replaced with an equal amount of normal rabbit serum; (lane D) standard protein marker, molecular weights indicated by arrows.

inactivated cytomegalovirus (Cx-90-3B) (13). Immunoprecipitates thus formed did not contain any significant amount of radioactivity when compared to the control (Fig. 2).

DISCUSSION

The proteins present on the surface of a virion are particularly interesting because some, or all, of these surface proteins are target antigens for neutralizing antibody. Analysis of [^{14}C]-glucosamine- or [^{14}C]fucose-labeled HSV-1 characterized 12 to 14 polypeptides in the range of 50,000 to 130,000 molecular weight in the virion envelope (12). This method of analysis does not distinguish between the glycoproteins present inside the envelope and those present outside. In contrast, analysis of surface-labeled virus by ^{125}I in the presence of lactoperoxidase and H_2O_2 indicates that the proteins are present on the surface of the virion and that they are accessible to the outer macromolecules. Thus, we found that at least six major and four minor proteins were present on the surface of the virion. The virus specificity of these surface glycoproteins was confirmed by the ability of hyperimmune antisera against HSV-1 to immunoprecipitate these proteins (Gupta and Rapp, manuscript in preparation). Furthermore, at least all major surface proteins are glycosylated, as indicated by their capacity to bind concanavalin A. Although similar surface glycoproteins in the range of 50,000 to 120,000 were detected in the analysis of both surface-labeled and glucosamine- or fucose-labeled virus, no glucosamine- or fucose-labeled protein of molecular weight below 50,000 was detected (12). The apparent discrepancy could be due to the difference in the host cell in which the virus was grown or the presence of a very low content of glucosamine or fucose in these polypeptides. Olshevsky and Becker (14) have reported a similar analysis by polyacrylamide gel electrophoresis of surface-labeled HSV-1 virions. Because of the lower resolution in their gel system, it is very difficult to compare results. However, one of the major surface proteins (molecular weight 93,000) detected in their analysis was present in very small amounts in our preparation. Both studies detected a major surface protein of an approximate molecular weight of 58,000. The differences noted may be due to the difference in methods of purification of the virus and the host cells used to grow the virus.

Using rabbit antisera to tumor 10, it was possible to selectively immunoprecipitate three major surface proteins of HSV-1, of which at least two are also probably part of the HSV-2 virion (data not shown). Thus, it was concluded that these virion structural proteins must be present in tumor 10. Since tumor 10 was derived by injecting the HSV-1-transformed cell line 14-012-8-1 into a hamster, it is logical to conclude that the transformed cells must also contain these three proteins. The finding of herpes virion proteins in these transformed cells is in agreement with the observations, with the same cells, that demonstrate virus-specific mRNA (Z. Ben-Ishai and L. Vardimon, personal communication).

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