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Shared Antigenic Determinants Between Two Distinct Classes of Proteins in Cells Infected with Herpes Simplex Virus

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Guinea pig antisera and mouse monoclonal antibodies against a 40,000-molecular-weight nucleocapsid protein (p40) of herpes simplex virus types 1 and 2 immunoprecipitated 40,000- and 80,000-molecular-weight classes of soluble proteins from infected cell extracts. The soluble 40,000-molecular-weight protein class (intracellular p40) appeared as a cluster of three to four closely spaced bands of proteins having molecular weights ranging between 39,000 and 45,000, whereas the soluble 80,000-molecular-weight protein class (intracellular p80) appeared as a doublet of bands. The peptide map of intracellular p40 closely resembled the maps of the p40 and p45 proteins of nucleocapsids, but it showed both differences and similarities when compared with the peptide map of intracellular p80. Pulse-chase experiments suggested that intracellular p80 was not a precursor of intracellular p40. We conclude that the intracellular p40 and p80 protein classes share common antigenic determinants, presumably reflecting similar amino acid sequences, although they have distinct differences in protein structure.

In our laboratory we have been engaged in studying the nucleocapsid proteins of herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2). We have shown by competition immunoassays that a 40,000-molecular-weight polypeptide, p40, which is a major component of these nucleocapsids, possesses both type-specific and cross-reactive antigenic determinants (7). Radioimmunoprecipitation studies with monospecific antisera and monoclonal antibodies have indicated that p40 shares at least some of these determinants with another nucleocapsid protein, protein p45 (14).

The finding that nucleocapsid proteins p40 and p45 share antigenic determinants led us to examine infected cells for the presence of other proteins which might have the same determinants. Such proteins might be precursors, polyproteins, or other types of derivatives which could indicate important relationships among these proteins.

We found that antibodies against nucleocapsid p40 immunoprecipitated 40,000- and 80,000-molecular-weight classes of proteins from infected cell extracts. The 40,000-molecular-weight class (intracellular p40) appeared to include the nucleocapsid proteins p40 and p45, whereas the 80,000-molecular-weight class (intracellular p80) was not found in nucleocapsid preparations. Pulse-chase experiments failed to show a precursor-product relationship between intracellular p80 and intracellular p40. The peptide maps of these two classes of proteins showed both differences and similarities.

MATERIALS AND METHODS

Cells, viruses, and antibody preparation. HSV-1 strain MAL and HSV-2 strain MS were grown in Vero cells as previously described (7). Guinea pig antisera against HSV-1 and HSV-2 p40's were prepared by immunizing animals with purified p40 protein obtained from polyacrylamide gels (7). Ascites fluids containing monoclonal antibodies against p40 were produced by intraperitoneal inoculations of hybrid cell lines 1D4 (which secretes anti-HSV-1 antibodies) and 3E1 (which secretes anti-HSV-2 antibodies) (14) into BALB/c mice primed with Pristane (Aldrich Chemical Co., Milwaukee, Wis.), as described by Lostrum et al. (9).

Purification of virions. Virions were purified essentially as described by Spear and Roizman (13). Cells infected with HSV-1 or HSV-2 were harvested 24 h postinfection, suspended in 2 volumes of 1 mM dibasic sodium phosphate (pH 8.2) containing 0.1 mM phenylmethylsulfonyl fluoride, and allowed to swell for 10 min at 0°C. The cells were disrupted by dounce homogenization, and the nuclei were pelleted by centrifugation at 800 × *g* for 10 min and stored at −70°C until used for the purification of nucleocapsids (see below). Debris was removed from the cytoplasmic fraction by centrifugation in a Sorvall SS34 rotor at 8,000 rpm for 5 min. The virions in the cytoplasm were sedimented by centrifugation at 60,000 × *g* for 1 h, suspended in 1 mM dibasic sodium phosphate, and subjected to sonic treatment. After incubation in the presence of DNase (50 μg/ml) and RNase (50 μg/ml) for 30 min at room temperature, the virion samples were layered onto 34-ml 5 to 30% (wt/vol) dextran T10 (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.) gradients in 1 mM dibasic sodium phosphate and centrifuged for 1 h at 20,000 rpm in a Beckman SW27 rotor. The virion-containing band was collected, di-

luted approximately fourfold with 10 mM Tris-hydrochloride (pH 7.6)-1 mM EDTA (TE buffer), and pelleted by centrifugation at 25,000 rpm for 1 h in a Beckman SW27 rotor. The virion pellet was suspended in a small volume of TE buffer and was layered onto an 11-ml 10 to 50% (wt/wt) potassium tartrate gradient in TE buffer. Centrifugation of the gradient was performed with a Beckman SW41 rotor for 2 h at 25,000 rpm. The virus band was collected, dialyzed against TE buffer, and stored at −70°C.

Purification of nucleocapsids. Nuclei of infected cells obtained as described above were suspended in about 2 volumes of 0.1 M Tris-hydrochloride (pH 8.0)-1.5 mM $MgCl_2$-0.1 mM phenylmethylsulfonyl fluoride. The nuclei were lysed by adding sodium deoxycholate to a final concentration of 0.5%, followed by sonic treatment. The nuclear lysates were incubated at room temperature for 30 min in the presence of 50 μg of DNase per ml; this was followed by centrifugation at 8,000 rpm for 5 min in a Sorvall SS34 rotor to remove debris. Nucleocapsids were purified from clarified nuclear lysates by centrifugation through 35% (wt/vol) sucrose, followed by centrifugation in a 10 to 40% (wt/wt) sucrose gradient, as described previously (6, 7, 13).

Radiolabeling of cells. Cells were washed once with methionine-free Eagle minimal essential medium containing 5% dialyzed heat-inactivated fetal calf serum and then labeled with 100 μCi of [^{35}S]methionine (800 to 1,200 Ci/mmol; Amersham Corp., Arlington Heights, Ill.) per ml in the same methionine-free medium for 1 to 4 h. The cell sheet was then washed twice with ice-cold Tris-buffered saline (pH 7.4) and scraped, and the cells were suspended in cold Tris-buffered saline. The cells were sedimented by centrifugation at 800 × *g* for 10 min, and the cell pellets were stored at −70°C until used.

Preparation of cell extracts and disrupted virus particles for immunoprecipitation. Cell extracts were prepared by suspending the [^{35}S]methionine-labeled cells in buffer A (0.1 M Tris-hydrochloride [pH 8.0], 10% [vol/vol] glycerol, 0.5% Nonidet P-40, 0.5% sodium deoxycholate, 0.2 mM phenylmethylsulfonyl fluoride) and incubating them for 1 h at 4°C with shaking. The extracts were clarified by centrifugation at 60,000 × *g* for 1 h. [^{35}S]methionine-labeled virions and nucleocapsids were disrupted by heating at 100°C for 5 min in 0.5% sodium dodecyl sulfate (SDS)-2.5% β-mercaptoethanol-0.05 M Tris-hydrochloride (pH 8.0), followed by a 10-fold dilution with buffer A (14).

Immunoprecipitation and SDS-polyacrylamide gel electrophoresis. Antibody (20 μl) was incubated with a 0.5-ml portion of either cell extract or disrupted virus particles for 3 h at 4°C and then further incubated for 1 h with 0.12 ml of a 33% (vol/vol) suspension of protein A-Sepharose CL-4B beads (Pharmacia Fine Chemicals, Inc.). After incubation, the beads were washed with 0.5 M LiCl-0.1 M Tris-hydrochloride (pH 8.0)-1% β-mercaptoethanol, suspended in an equal volume of 2% SDS-20% glycerol-5% β-mercaptoethanol-0.125 M Tris-hydrochloride (pH 6.8)-0.004% bromophenol blue, and heated at 100°C for 5 min, as previously described (14). The proteins were then separated by electrophoresis on a 5 to 20% polyacrylamide gel gradient containing SDS (7), and autoradiographs or fluorographs were prepared on Kodak SB-5 X-ray film (1). The absorbances at 595 nm of the bands in the autoradiographs and fluorographs were measured with a scanning densitometer (Transidyne General Corp., Ann Arbor, Mich.)

Peptide mapping. Proteins obtained from polyacrylamide gels were partially digested with *Staphylococcus aureus* V8 protease (Miles Laboratories, Inc., Elkhart, Ind.), and the peptides were analyzed by SDS-polyacrylamide gel electrophoresis essentially as described by Cleveland et al. (4) and Bordier and Crettol-Järvinen (2). After separation by electrophoresis, [^{35}S]methionine-labeled proteins were located in dried unfixed gels by alignment with autoradiographs, and rectangular segments (0.5 by 1.5 cm) of lanes containing these proteins were excised from the gel slabs and incubated in 0.125 M Tris-hydrochloride (pH 6.8)-0.1% SDS-1 mM EDTA for 30 min at room temperature. The gel sections were drained and placed in sample wells (width, 2 cm) of second-dimension polyacrylamide gels, which were composed of a 7-cm 5 to 20% gradient gel beneath a 3-cm 4% stacking gel. The gel segments were oriented so that their bands were parallel to the direction of electrophoresis in the second-dimension gel, and then they were covered with melted (55°C) 1% agarose in 0.125 M Tris-hydrochloride (pH 6.8)-0.1% SDS-1 mM EDTA. *S. aureus* V8 protease in 0.125 M Tris-hydrochloride (pH 6.8)-0.1% SDS-1 mM EDTA-10% glycerol-0.004% bromophenol blue was overlaid on the hardened agarose gel, and electrophoresis was conducted at low voltage (25 V) for 20 h to allow partial proteolytic digestion to occur during the stacking phase of electrophoresis. After electrophoresis, the gels were processed for fluorography on Kodak SB-5 X-ray film by the method of Bonner and Lasky (1).

Competition immunoassays. Purified virions and nucleocapsids were disrupted in 0.01 M Tris-hydrochloride (pH 7.8)-0.01 M NaCl-0.1% Triton X-100 containing 1% SDS and 5 mM dithiothreitol by heating at 100°C for 5 min. The preparations were tested at serial twofold dilutions in the same buffer without SDS or dithiothreitol for ability to compete with ^{125}I-labeled HSV-1 strain MAL nucleocapsid p40 (10,000 cpm) for binding limiting concentrations of guinea pig antiserum against HSV-1 nucleocapsid p40. Antiserum was used at a dilution of 1:400, which precipitated approximately 35% of the ^{125}I-labeled p40. Preparations of ^{125}I-labeled p40, the antiserum, and the reaction conditions have been described previously (7).

RESULTS

Reaction of guinea pig antiserum with cell extracts. HSV-2-infected and uninfected cells labeled with [^{35}S]methionine were disrupted with detergents and were clarified by high-speed centrifugation, which sedimented virus particles and insoluble proteins. A large number of soluble virus-specified proteins were observed in infected cell extracts by SDS-polyacrylamide gel electrophoresis (Fig. 1, lane A), as evidenced by their absence in uninfected cell

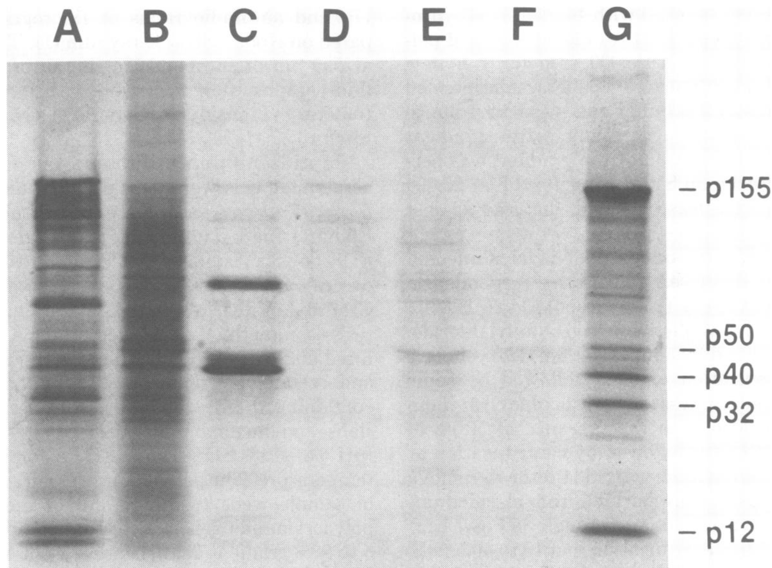

FIG. 1. *SDS-polyacrylamide gel electrophoresis of soluble cell extract proteins immunoprecipitated by guinea pig antiserum against HSV-2 nucleocapsid p40. HSV-2- and mock-infected cells were labeled with [^{35}S]methionine between 18 and 22 h postinfection. Extracts were prepared and reacted with either guinea pig antiserum against HSV-2 nucleocapsid p40 or control guinea pig serum. Proteins from cell extracts, immunoprecipitates, and nucleocapsids were separated by electrophoresis, and an autoradiograph was prepared. Lane A, proteins of HSV-2-infected cell extracts; lane B, the proteins of mock-infected cell extracts; lanes C and D, proteins precipitated from HSV-2-infected cell extracts by guinea pig antiserum against HSV-2 nucleocapsid p40 (lane C) and by control nonimmune guinea pig serum (lane D); lanes E and F, proteins precipitated from mock-infected cell extracts by guinea pig antiserum against HSV-2 nucleocapsid p40 (lane E) and by control nonimmune guinea pig serum (lane F); lane G, proteins of purified [^{35}S]methionine-labeled HSV-2 nucleocapsids. The major nucleocapsid proteins are designated at the right of the autoradiograph. The p45 band is the minor band immediately beneath p50.*

extracts (lane B). Guinea pig antiserum against the HSV-2 nucleocapsid p40 protein immunoprecipitated two classes of viral proteins from infected cell extracts (lane C); these were intracellular p80, which appeared as a band doublet, and intracellular p40, which appeared as three or four closely spaced proteins encompassing a molecular weight range between 39,000 and 45,000. This antiserum did not precipitate these protein classes from uninfected cell extracts (lane E), and nonimmune guinea pig serum did not precipitate them from either infected (lane D) or uninfected (lane F) cell extracts. Comparable results were obtained with HSV-1-infected cell extracts and antiserum against HSV-1 p40 (see below). Labeled proteins were not immunoprecipitated from infected cells incubated with [^{14}C]glucosamine, suggesting that intracellular p40 and p80 were not glycoproteins (data not shown).

Synthesis of intracellular p40 and p80 after infection. In an experiment in which cells were pulse-labeled with [^{35}S]methionine at intervals after HSV-2 infection, synthesis of intracellular p40 and p80 was first detected by immunoprecipitation with guinea pig antiserum at about 3 h postinfection (Fig. 2). The rate of production of these protein classes remained roughly constant between 6 and 24 h postinfection, but considerably less intracellular p80 was usually precipitated at 24 h postinfection. Although the cause of this reduction in precipitable intracellular p80 is uncertain, substantial cytopathic effects were evident at this late stage in infection, and intracellular p80 may have been selectively lost into the medium or degraded by proteases. Antiserum was reacted with extracts of infected cells which were pulse-labeled for 15 min and then chased for up to 4 h with isotope-free medium (Fig. 3). SDS-polyacrylamide gel electrophoresis showed that the ratio of the intensities of the bands of intracellular p40 and p80 remained constant during the chase period, suggesting that intracellular p40 and p80 did not serve as precursors to one another.

Reaction of guinea pig antiserum with purified proteins. To verify that intracellular p40 and p80 possess common antigenic determinants, each protein class was purified from polyacrylamide gels and was successfully immunoprecipitated by guinea pig antiserum against nucleocapsid p40 (Fig. 4). Therefore, it

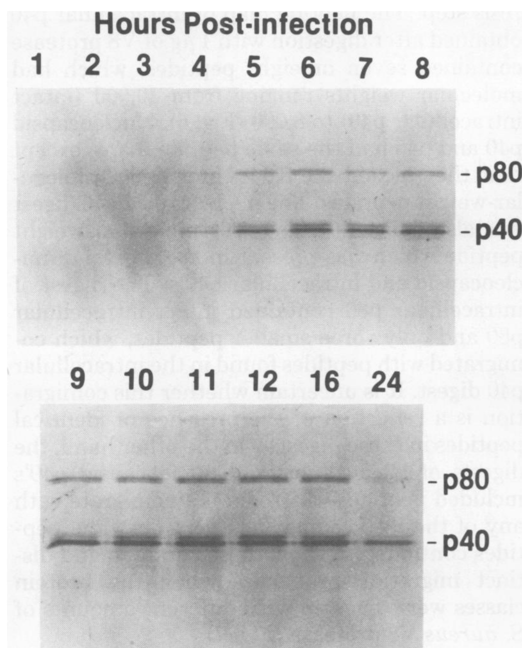

FIG. 2. *Synthesis of intracellular p40 and p80 after HSV-2 infection. Cells were labeled with [^{35}S]methionine for 30 min, terminating at the indicated times after infection. Cell extracts were prepared and reacted with guinea pig antiserum against HSV-2 nucleocapsid p40. The proteins in the immunoprecipitates were separated by SDS-polyacrylamide gel electrophoresis, and an autoradiograph was made.*

is unlikely that the precipitation of intracellular p80 from infected cell extracts was due to specific or nonspecific binding to intracellular p40. Furthermore, although the p40 protein exists in disulfide-linked complexes in nucleocapsids (13), soluble disulfide-linked complexes containing intracellular p40 or p80 were not detected by nonreducing SDS-polyacrylamide gel electrophoresis (data not shown).

Reaction of monoclonal antibodies with infected cell extracts. Recently, we established mouse hybrid cell lines which synthesize monoclonal antibodies against the p40 and p45 proteins of HSV-1 and HSV-2 nucleocapsids (14). Ascites fluids containing high titers of monoclonal antibodies were prepared and were reacted with extracts of cells infected with either HSV-1 or HSV-2. The anti-HSV-1 p40 monoclonal antibody produced by cell line 1D4 precipitated intracellular p40 and p80 from only HSV-1-infected cell extracts (Fig. 5A). In contrast, the anti-HSV-2 p40 monoclonal antibody produced by cell line 3E1 precipitated both HSV-1 and HSV-2 intracellular p40 and p80 (Fig. 5B), although the homologous proteins were precipitated at higher dilutions of 3E1 antibody than were the heterologous proteins. Guinea pig antisera against the nucleocapsid p40 proteins of HSV-1 and HSV-2 also precipitated homologous proteins at higher antibody dilutions, but the differences in the dilutions which precipitated the homologous and heterologous proteins were not as great as the difference observed with the 3E1 monoclonal antibody (Fig. 5A and B).

Peptide maps of immunoprecipitated proteins. The finding that monospecific antisera and monoclonal antibodies reacted with HSV polypeptides having different molecular weights indicated that these polypeptides pos-

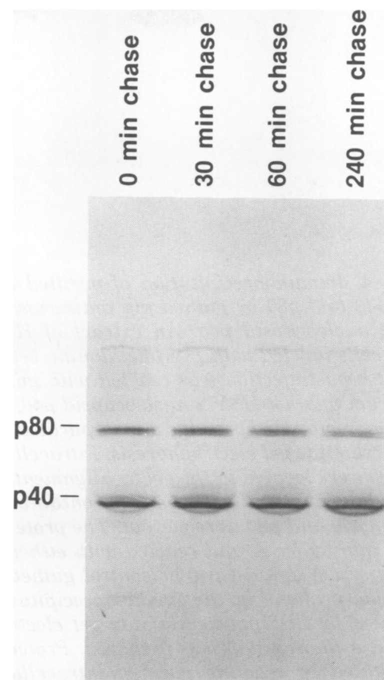

FIG. 3. *Autoradiogram of a polyacrylamide gel showing the immunoprecipitation of intracellular p40 and p80 from cells pulse-labeled with [^{35}S]methionine and chased in nonradioactive medium. At 16 h after HSV-2 infection, cells were incubated for 15 min in methionine-free medium. The medium was then removed, and the cells were pulse-labeled for 15 min with [^{35}S]methionine in fresh methionine-free medium. After the cells were washed three times and incubated (chased) in complete nonradioactive medium for the indicated periods of time, cell extracts were prepared and incubated with guinea pig antiserum against HSV-2 nucleocapsid p40. The immunoprecipitated proteins were separated by SDS-polyacrylamide gel electrophoresis, and autoradiographs were prepared. The relative intensities of the bands were measured on scanning densitometer tracings of autoradiographs prepared after varying intervals of exposure.*

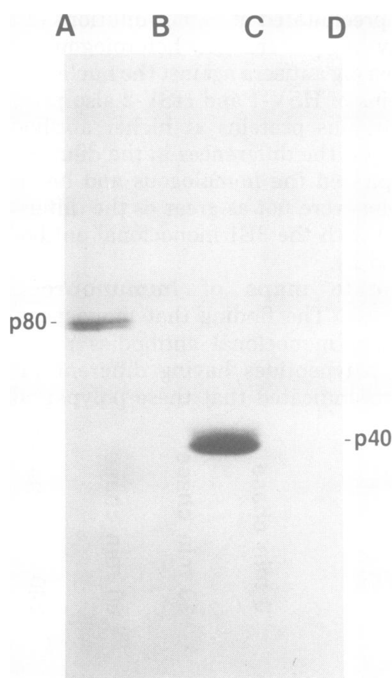

FIG. 4. Immunoprecipitation of purified intracellular p40 and p80 by guinea pig antiserum against HSV-2 nucleocapsid p40. An extract of HSV-2-infected cells labeled with [^{35}S]methionine between 18 and 22 h postinfection was reacted with guinea pig antiserum against HSV-2 nucleocapsid p40, and the immunoprecipitated proteins were separated by SDS-polyacrylamide gel electrophoresis. Intracellular p40 and p80 were located in the gel by alignment with an autoradiograph, and gel segments containing intracellular p40 and p80 were cut out. The proteins were eluted into buffer A and reacted with either guinea pig antiserum against p40 or control guinea pig serum. The proteins in the immunoprecipitates were separated by SDS-polyacrylamide gel electrophoresis, and a fluorograph was prepared. Proteins were precipitated by reacting purified intracellular p80 with antiserum against p40 (lane A) and control serum (lane B) and by reacting purified intracellular p40 with antiserum against p40 (lane C) and control serum (lane D).

sess common amino acid sequences. We tested this hypothesis by an analysis of the peptide maps of immunoprecipitated proteins after separation by SDS-polyacrylamide gel electrophoresis, partial digestion with *S. aureus* V8 protease, and resolution of the peptides by electrophoresis in a second SDS-polyacrylamide gel (2, 4). The peptide maps of the intracellular p40 and p80 classes represent the sums of the protein bands comprising each class, since the very similar mobilities of the bands prevented their clear resolution and separation in the first electrophoresis step. The peptide map of intracellular p40 obtained after digestion with 1 μg of V8 protease contained seven or eight peptides, which had molecular weights ranging from 40,000 (intact intracellular p40) to 8,000 (Fig. 6). Nucleocapsid p40 and p45 had the same peptide maps, except that they lacked the 8,000- and 11,000-molecular-weight peptides. The nucleocapsid p45 digest was also deficient in a 14,000-molecular-weight peptide which was present in the digests of nucleocapsid and intracellular p40's. The digest of intracellular p80 contained intact intracellular p80 and only three smaller peptides, which comigrated with peptides found in the intracellular p40 digest. It is uncertain whether this comigration is a reflection of the presence of identical peptides in these digests. On the other hand, the digests of intracellular and nucleocapsid p40's included peptides which did not comigrate with any of the intracellular p80 peptides. The peptides continued to show these common and distinct migration properties when the protein classes were digested with different amounts of *S. aureus* V8 protease (Fig. 7).

Reaction of antibodies with virion proteins. Virions of HSV-1 and HSV-2 were purified from cytoplasmic extracts by centrifugation in dextran T10 and potassium tartrate gradients. Electron microscope observations indicated that the HSV-1 virion preparations contained about 10 to 15% unenveloped nucleocapsids, whereas the HSV-2 preparations contained as many as 50% unenveloped particles. These findings are reasonably consistent with those obtained by Cassai et al. (3). Because of their higher purity, we concentrated our efforts on analyzing preparations of HSV-1 virions to determine whether they possess polypeptides immunologically related to p40. We were not able to identify directly nucleocapsid p40 and p45 in virions with certainty by electrophoretic analysis because of the presence of proteins having a migration similar to that of nucleocapsid p40 (Fig. 8). Guinea pig antiserum and mouse monoclonal antibody against HSV-1 nucleocapsid p40 precipitated much smaller amounts of nucleocapsid p40 and p45 from virion preparations than from preparations of nucleocapsids, whereas a protein having the mobility of intracellular p80 was not precipitated from either virion or nucleocapsid preparations. We were not able to compare accurately the amounts of p40 and p45 present in virions with the amounts found in nucleocapsids because the preparations may have had differing amounts of contaminating extraneous proteins.

DISCUSSION

Through the use of monoclonal antibodies and

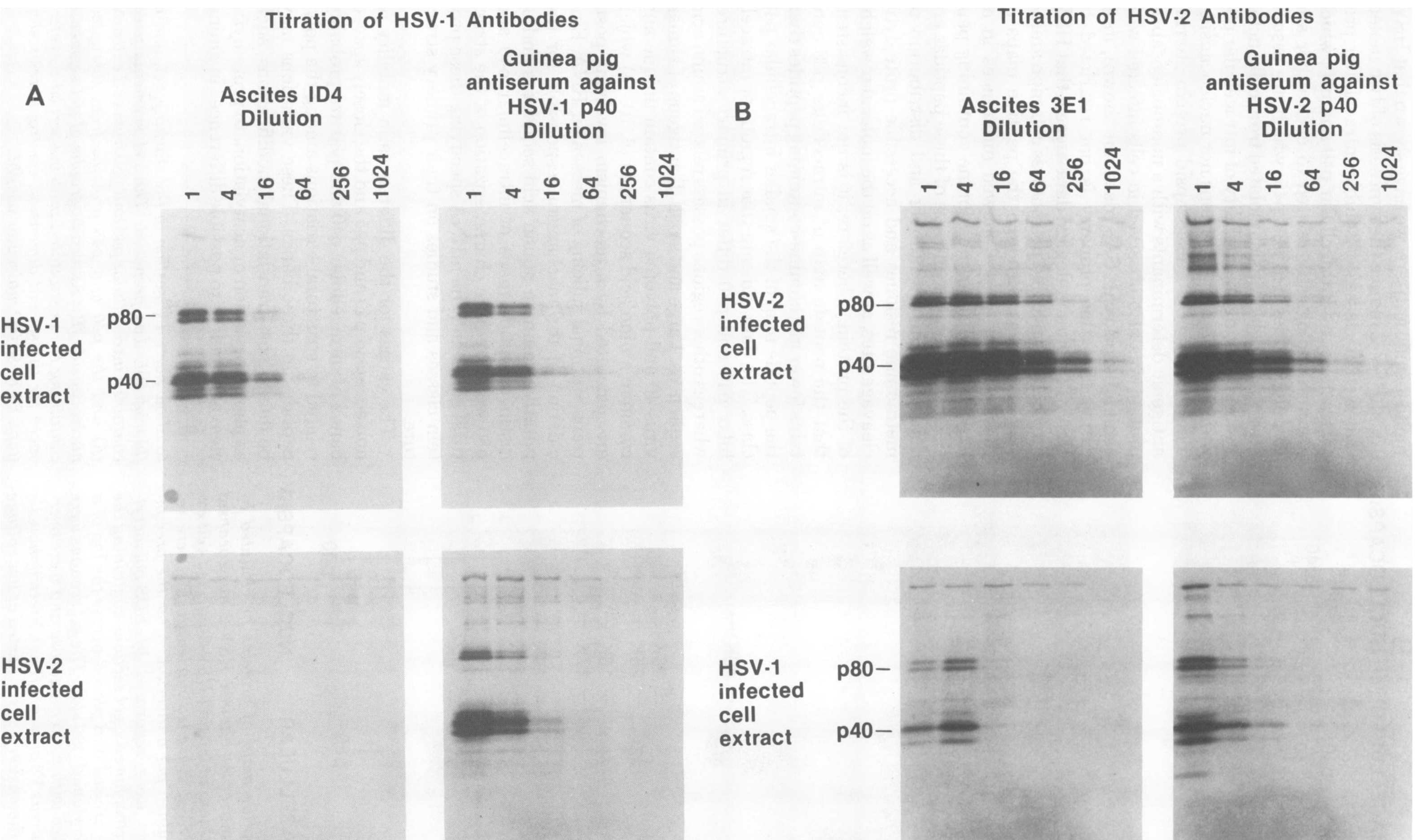

FIG. 5. *Immunoprecipitation of infected cell extracts with guinea pig antisera and monoclonal antibodies made against HSV-1 (A) and HSV-2 (B) nucleocapsid p40's. Dilutions of guinea pig antisera and monoclonal antibodies were reacted with extracts of [^{35}S]methionine-labeled cells labeled between 18 and 22 h after infection with either HSV-1 or HSV-2. The precipitated proteins were separated by SDS-polyacrylamide gel electrophoresis, and autoradiographs were prepared.*

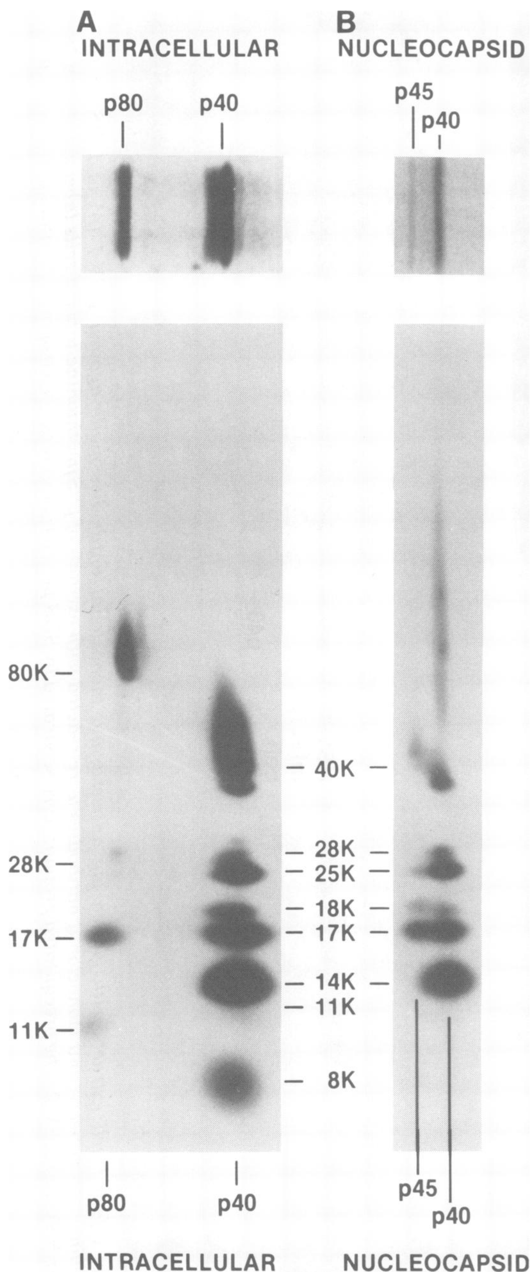

FIG. 6. *Peptide maps of proteins precipitated by guinea pig antiserum against HSV-2 nucleocapsid p40. [^{35}S]methionine-labeled HSV-2-infected cell extracts and HSV-2-disrupted nucleocapsids were reacted with guinea pig antiserum. After the precipitated proteins were separated by SDS-polyacrylamide gel electrophoresis, gel sections containing the proteins were excised and placed across the top of a second polyacrylamide gel slab. The gel sections were fixed into place with molten agarose and were overlaid with S. aureus V8 protease (1 μg). Electrophoresis in the second dimension was conducted at low voltage to allow partial digestion in the stacking gel, and a fluorograph was prepared. The gel segments used contained intracellular p40 and p80 from cell extracts (A) and the p40 and p45 proteins from disrupted nucleocapsids (B). Autoradiographs of gel segments containing the precipitated proteins served as references for the second-dimension electrophoresis step (top). 80 K, 80,000 molecular weight.*

monospecific antisera, we have shown that HSV-1 and HSV-2 induce the synthesis of two molecular weight classes of soluble intracellular proteins; these are intracellular p40 and p80, which are immunologically related to each other and to polypeptides p40 and p45, which are associated with nucleocapsids isolated from cell nuclei. Proteins having the mobility of intracellular p80 were found in neither virions nor nucleocapsids. The finding of a nucleocapsid protein sharing antigenic determinants with a non-nucleocapsid protein was also observed in cells infected with simian herpesvirus SA8 (data not shown), indicating that this property is not restricted to HSV. The immunological data suggest that HSV intracellular p40 and p80 possess common amino acid sequences, although the peptide maps of these protein classes showed differences in at least some of their methionine-containing peptides. The number and size of these peptides are dependent on the number and distribution of methionine residues and accessible proteolytic cleavage sites, as well as on the molecular weight of the protein. Therefore, it is not inconsistent that the peptide map of intracellular p80 contains fewer methionine-containing peptides than the peptide map of the smaller intracellular p40 class. The data indicate that regions of intracellular p40 and p80 differ in primary structure, whereas other regions possess related amino acid sequences. Large and small T antigens of simian virus 40 and polyoma virus contain unique and common amino acid sequences (8, 10, 11) which are generated by a mechanism involving post-transcriptional splicing of messenger RNA. Elucidation of the mechanism responsible for the putative common amino acid sequences and of distinct peptide maps of intracellular p40 and p80 would require characterization of the structural genes and the mRNA specifying these protein classes and studies on their primary structure.

The causes for the differences in mobility of nucleocapsid p40 and p45 and the closely spaced bands of intracellular p40 and p80 are probably related to structural variations among the polypeptides in these bands. These variations may be due to post-translational modifications, such as phosphorylation or acetylation, to proteolytic cleavage, and/or to possible differences in amino

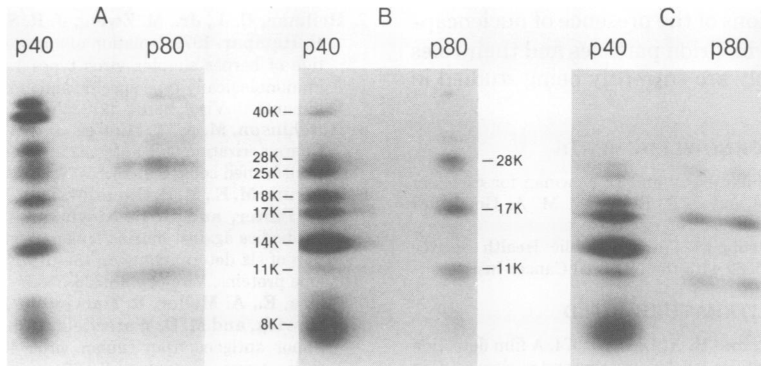

FIG. 7. *Peptide maps of HSV-2 intracellular p40 and p80. Peptide mapping was performed as described in the text, except that the gel segments from the first electrophoresis step were oriented so that the bands were perpendicular to the direction of the second electrophoresis step. The purified proteins were partially digested with 0.1 μg (A), 0.5 μg (B), and 1 μg (C) of S. aureus V8 protease. 40 K, 40,000 molecular weight.*

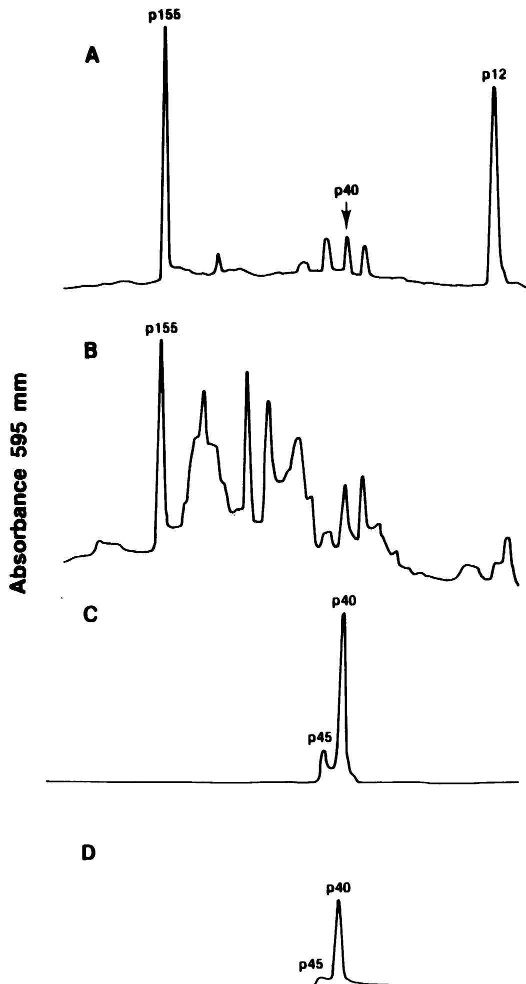

FIG. 8. *SDS-polyacrylamide gel electrophoresis of polypeptides of HSV-1 nucleocapsid and virion preparations immunoprecipitated by guinea pig antiserum against HSV-1 nucleocapsid p40. Virions and nucleocapsids were purified from cells labeled with [^{35}S]methionine between 18 and 22 h after HSV-1 infection. The labeled virus particles (100,000 cpm) were dissociated and reacted with guinea pig antiserum against HSV-1 nucleocapsid p40. Proteins of nucleocapsids, virions, and immunoprecipitates were separated by electrophoresis. Scanning densitometer tracings of autoradiograms and fluorograms were prepared. Autoradiogram tracings show polypeptides of HSV-1 nucleocapsids (A) and virions (B) after a 13-day exposure. Fluorogram tracings show polypeptides precipitated by guinea pig antiserum against HSV-1 nucleocapsid p40 from disrupted HSV-1 nucleocapsids after a 13-day exposure (C) and virions after a 40-day exposure (D).*

acid sequences in portions of the polypeptide chains. The peptide mapping studies indicate that the predominant polypeptide species of intracellular p40 is closely related to nucleocapsid p40 and p45. However, intracellular p40 also possesses polypeptides which are structurally different from nucleocapsid p40, since under the same conditions of partial proteolytic digestion, the digest of intracellular p40 contained peptides that were absent in the nucleocapsid p40 and p45 digests. The reasons for the appearance of these peptides have not yet been determined.

Little is known about the biochemistry of herpesvirus assembly. Studies employing electron microscopy indicate that nucleocapsids are assembled in the nucleus and are then enveloped during passage through the nuclear membrane to form complete infectious particles, which are found in the cytoplasm (5). Although the function of intracellular p80 remains unknown, we believe that intracellular p40 participates in virus assembly, since the p40 and p45 proteins are major constituents of intranuclear nucleocap-

sids. The questions of the presence of nucleocapsid p40 and p45 in virion particles and their roles in virus assembly are currently being studied in this laboratory.

ACKNOWLEDGMENTS

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