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Herpes Simplex Virus Type 1 and 2 Intracellular p40: Type-Specific and Cross-Reactive Antigenic Determinants on Peptides Generated by Partial Proteolysis

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Intracellular p40 is a class of protein ranging in molecular weight from 39,000 to 45,000 that is immunoprecipitated from herpes simplex virus type 1 (HSV-1)- and HSV-2-infected cell extracts by mouse monoclonal antibodies or guinea pig antisera against HSV-1 and HSV-2 nucleocapsid p40. Analysis by a two-dimensional gel system showed that HSV-1 and HSV-2 intracellular p40 each consisted of three major components. However, these HSV-1 and HSV-2 proteins differed in charge and size. Analysis of *Staphylococcus aureus* V8 protease partial digests by two-dimensional gel electrophoresis indicated that none of the peptides of HSV-1 and HSV-2 intracellular p40 were identical. Immunoprecipitation of the partial digest products of intracellular p40-1 and p40-2 with homologous and heterologous guinea pig antisera resulted in the precipitation of various combinations of peptides indicating the presence of either type-specific or cross-reactive antigenic determinants.

Cells productively infected with herpes simplex virus type 1 (HSV-1) or HSV-2 synthesize 50 or more virus-induced polypeptides (6, 11), some of which contain cross-reactive antigenic determinants (5, 12, 13, 15). Type-specific and cross-reactive antigenic determinants have been detected on individual viral proteins, including glycoprotein D of HSV-1 (gD-1) (10, 14; Showalter, Zweig, and Hampar, Infect. Immun., in press), and the nucleocapsid-associated 40,000-dalton proteins of HSV-1 and HSV-2 (nucleocapsid p40-1 and p40-2, respectively [4]). Immunoprecipitation studies with monoclonal antibodies and guinea pig antisera led to the identification of other viral polypeptides that are immunologically related to nucleocapsid p40-1 and p40-2. These include a minor nucleocapsid-associated 45,000-dalton protein (17) as well as 40,000- and 80,000-dalton classes of soluble intracellular proteins found in infected cell extracts (16). The 40,000-dalton class of proteins from HSV-1- and HSV-2-infected cells (intracellular p40-1 and p40-2, respectively) were previously identified as clusters of closely spaced bands. The peptide map of intracellular p40-2 is similar to that of nucleocapsid p40-2 (16). The aim of this study was to further characterize the physical and immunological properties of the proteins which comprise intracellular p40-1 and p40-2.

We found that both intracellular p40-1 and p40-2 resolved into three major protein components after separation in a two-dimensional gel system. However, the protein patterns and the peptide maps of intracellular p40-1 and p40-2 were distinct. Immunoprecipitation of V8 protease digest peptides of intracellular p40-1 and p40-2 with guinea pig antisera showed that some peptides contain type-specific antigenic determinants, whereas others contain cross-reactive antigenic determinants.

MATERIALS AND METHODS

Cells, viruses, and antibody preparations. HSV-1 strain MAL and HSV-2 strain MS were grown in Vero cell cultures as previously described (4). Antibodies were prepared against disrupted HSV-2 MS nucleocapsids purified as previously described (4). Nucleocapsids were disrupted in 1% (wt/vol) sodium dodecyl sulfate (SDS) and 0.1% β-mercaptoethanol, heated at 100°C for 5 min, and then diluted fivefold with phosphate-buffered saline and mixed with an equal volume of Freund adjuvant. Guinea pigs were given three weekly intramuscular injections of disrupted nucleocapsids (100 μg per inoculation), and sera were obtained 7 days after the last injection. Complete Freund adjuvant was used in the first injection, and incomplete adjuvant was used in the two subsequent inoculations. Guinea pig antisera against HSV-1 and HSV-2 nucleocapsid p40 and a simian herpesvirus SA8 nucleocapsid protein designated p30 were prepared by inoculating SDS-polyacrylamide gel fragments containing these proteins (4). Ascites fluids, containing monoclonal antibodies against nucleocapsid p40-1 and

p40-2, were obtained from BALB/c mice which developed tumors after inoculation with hybrid cell lines 1D4 (anti-p40-1 antibody) or 3E1 (anti-p40-2 antibody) (17).

Radiolabeling of cells and preparation of cell extracts for immunoprecipitation. Vero cell cultures were infected with HSV-1 MAL or HSV-2 MS at a multiplicity of infection of 5. After virus adsorption for 1 h at 37°C, fresh medium was added, and the cultures were maintained at 37°C. Infected cell cultures were washed once with methionine-free Eagle minimum essential medium containing 5% dialyzed fetal calf serum (heat inactivated) and then labeled for 4 to 6 h 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 Eagle minimum essential medium. Cells were washed with ice-cold Tris-buffered saline and then scraped into buffer A (0.1 M Tris-hydrochloride [pH 8.0] containing 0.5% Nonidet P-40, 0.5% sodium deoxycholate, 10% [vol/vol] glycerol, 2% ethanol, and 0.2 mM phenylmethylsulfonyl fluoride). After incubation for 1 h at 4 °C with shaking, cell extracts were clarified by centrifugation at 60,000 × *g* for 1 h. Clarified extracts were either used immediately or stored at −70°C.

Immunoprecipitation and SDS-PAGE. Antibody was reacted with antigen (10 μl of antibody per 0.5 ml of cell extract) for 90 min at 4°C with shaking before the addition of 100 μl of a 33% (vol/vol) suspension of protein A–Sepharose CL-4B (Pharmacia Fine Chemicals, Inc., Piscataway, N.J.) in buffer A. After mixing for 1 h at 4°C, immune precipitates were washed and prepared for SDS-polyacrylamide gel electrophoresis (PAGE) as previously described (16). Proteins were separated on SDS-containing 5 to 20% polyacrylamide gradient gels in a Bio-Rad model 220 electrophoresis apparatus (4), and protein bands were visualized by autoradiography or fluorography on Kodak SB-5 X-ray film. Fluorographs were prepared by fixing the gel in 20% (vol/vol) 2-propanol–10% (vol/vol) acetic acid for 1 h, followed by a 2-h incubation in En^{3}Hance (New England Nuclear Corp.) and 1 h in distilled water. Gels were then dried onto filter paper with a Bio-Rad model 224 slab gel dryer.

Purification of intracellular p40. [^{35}S]methionine-labeled, infected cell extracts were reacted with homologous monoclonal antibody against nucleocapsid p40, and the proteins immunoprecipitated were subsequently separated by SDS-PAGE. Wet gels were wrapped in Saran Wrap and then taped in place in a cardboard cassette on Kodak SB-5 X-ray film. After 16 to 24 h of exposure the autoradiographs were developed, the gels were aligned with the film, and intracellular p40 bands were excised, minced, and transferred to 1.0 ml of either buffer A or 0.1% SDS. After the proteins were extracted for 24 h at 4°C, the supernatant fluid was removed and clarified at 12,800 × *g* (Eppendorf centrifuge model 5412) for 15 min and then stored at −70°C. The above extraction procedure was repeated until the majority of the protein was eluted from the gel.

Proteolytic cleavage of intracellular p40. Purified [^{35}S]methionine-labeled intracellular p40 proteins were partially digested by a modification of the Cleveland technique (3). Proteolysis was conducted for 30 min at 37°C in the presence of 50 μl of solutions containing 2.5 to 25 μg of *Staphylococcus aureus* V8 protease (Miles Laboratories, Inc.) per 10,000 cpm of protein. For peptide map analysis, the proteins were digested with enzyme concentrations ranging from 0.05 to 500 μg/ml in buffer A. Two-dimensional gel analysis and immunoprecipitation studies were conducted by using the partial digest products of intracellular p40-1 and p40-2 after exposure to 50 μg of V8 protease per ml in buffer A. After proteolysis the reaction mixture was diluted twofold in buffer A containing 0.4 mM phenylmethylsulfonyl fluoride, followed by a 30-min incubation in an ice bath.

Two-dimensional gel electrophoresis. [^{35}S]methionine-labeled intracellular p40-1 and p40-2 proteins (intact) were eluted from wet gels into 0.1% SDS. Each sample (10,000 cpm of protein in 3 to 5 μl of eluent) was mixed with 20 μl of the lysis buffer described by O'Farrel and O'Farrel (9). Focusing gels (10 cm in length) were prepared in Pyrex tubes (internal diameter, 3.0 mm) by the method of O'Farrel and O'Farrel (9), with the exception that 1.6% ampholines at pH 6 to 8 were used in place of ampholines at pH 5 to 7. After prefocusing of the gels, samples were loaded, and isoelectric focusing was carried out at 300 V for 18 h and 800 V for 1 h. Gels were removed from the tubes, incubated for 1 h at room temperature in SDS-sample buffer (1% SDS, 10% glycerol, 2.5% β-mercaptoethanol, 0.0625 M Tris-hydrochloride [pH 6.7], 0.002% bromophenol blue), and either prepared immediately for protein separation in the second dimension or stored at −70°C. Frozen gels were quickly thawed in warm running water in preparation for electrophoresis in the second dimension. The isoelectric focusing gel was immobilized in 1% agarose containing 0.125 M Tris-hydrochloride (pH 6.7) and 0.1% SDS above a 5% (wt/vol) polyacrylamide stacking gel. SDS-gel electrophoresis was conducted in a Hoefer model SE-600 slab gel apparatus for 19 h at 45 V in a discontinuous buffer system containing a linear 5 to 20% (wt/vol) polyacrylamide gradient (7). The stacking gel was removed, and the resolving gels were processed for fluorography. V8 protease digests of intracellular p40-1 or p40-2 were mixed with an equal volume of lysis buffer and then layered onto 11.5-cm isoelectric focusing gels prepared by a modification of the protocol of Breithaupt et al. (2). The same gel recipe was followed, with the exception that LKB ampholytes at pH 3.5 to 10 (0.6 ml) and pH 7 to 9 (0.4 ml) were used. Gels were overlaid with water until polymerized and overlaid with lysis buffer and subsequently treated and run as described by O'Farrel and O'Farrel (9). After isoelectric focusing, the gels were equilibrated in SDS sample buffer and separated in second-dimension, SDS-polyacrylamide gels as described above.

RESULTS

Immunoprecipitation of intracellular p40-1 and p40-2 proteins. [^{35}S]methionine-labeled, HSV-1- and HSV-2-infected cell extracts were reacted with guinea pig antiserum to nucleocapsid p40-1, which immunoprecipitates nucleocapsid and intracellular proteins immunologically related to nucleocapsid p40-1 (16).

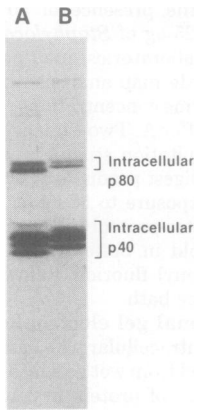

FIG. 1. *Autoradiogram after SDS-PAGE of soluble [^{35}S]methionine-labeled, infected cell extracts immunoprecipitated with guinea pig antiserum to nucleocapsid p40-1. HSV-1- and HSV-2-infected cells were labeled with [^{35}S]methionine from 16 to 22 h postinfection. Cell extracts were prepared and clarified. Lanes: A, proteins immunoprecipitated from HSV-1-infected cell extracts; B, proteins immunoprecipitated from HSV-2-infected cell extracts.*

Comparative SDS-PAGE of immune precipitates (Fig. 1) was conducted 2 to 4 h longer (18 to 20 h) than in previous experiments (4) to obtain greater resolution of the component intracellular p40-1 and p40-2 proteins. The guinea pig antiserum immunoprecipitated both the 40,000-dalton (intracellular p40) and 80,000-dalton (intracellular p80) classes of protein from HSV-1-infected (Fig. 1, lane A) and HSV-2-infected (Fig. 1, lane B) cells. The molecular mass estimate of intracellular p80 varied between 70,000 and 80,000 daltons in different gels, whereas intracellular p40 consistently migrated to the 40,000-dalton region. The reason for this variability in electrophoretic migration of p80 has not been determined. Identical SDS-gel patterns were obtained when these cell extracts were reacted with guinea pig antiserum to nucleocapsid p40-2 or with the homologous monoclonal antibody to the respective nucleocapsid p40 protein (data not shown). Intracellular p40-1 (Fig. 1, lane A) resolved into three major bands, whereas intracellular p40-2 (Fig. 1, lane B) resolved into only two major bands. Close examination revealed that each of the major intracellular p40-1 or p40-2 constituents migrates to a distinct position in the gel.

Two-dimensional gel analysis of intracellular p40-1 and p40-2 proteins. To further characterize and resolve the polypeptides of intracellular p40-1 and p40-2, these proteins were subjected to separation by two-dimensional gel electrophoresis. [^{35}S]methionine-labeled, HSV-1- and HSV-2-infected cell extracts were preparatively immunoprecipitated with homologous monoclonal antibody to nucleocapsid p40 (17). After separation of intracellular p80 and intracellular p40 by SDS-PAGE, intracellular p40 bands were excised from the gel, eluted into 0.1% SDS, and diluted into lysis buffer. The presence of the nonionic detergent Nonidet P-40 in the lysis buffer causes the SDS to separate from the protein and form micelles with the Nonidet P-40, thereby allowing separation of the proteins by charge (1, 8). Intracellular p40 proteins were prepared in this manner instead of by directly analyzing immune precipitates of intracellular p40 and p80 so that the intact proteins and their partial digest products (see below) would be analyzed under the same conditions.

The three major intracellular p40-1 bands shown in Fig. 1 resolved into three major spots with isoelectric points ranging from 6.52 to 6.63 (Fig. 2A). The two major intracellular p40-2 components observed by single-dimensional SDS-PAGE resolved into three major proteins by two-dimensional separation (Fig. 2B), indicating that the slower-migrating intracellular p40-2 band contains two overlapping proteins which have different net charges. The isoelectric points of the intracellular p40-2 bands range from 6.80 to 6.89. The difference in the isoelectric points of intracellular p40-1 proteins from intracellular p40-2 proteins allowed clear separation of these proteins even when they were analyzed as a mixture in the same gel (Fig. 2C).

Comparison of the partial V8 protease cleavage products of intracellular p40-1 and p40-2. Based on the immunological data that nucleocapsid and intracellular p40 proteins of HSV-1 and HSV-2 share cross-reactive and type-specific antigenic determinants (4), one might expect to see similarities and differences in their peptide maps after partial proteolytic cleavage. After digesting intracellular p40-1 and p40-2 with various concentrations of V8 protease, their respective [^{35}S]methionine-containing partial digest products were compared by SDS-PAGE (Fig. 3). Under these experimental conditions, significant proteolysis of intracellular p40-1 and p40-2 occurred after exposure to V8 protease concentrations between 5.0 and 500 μg/ml. The intracellular p40-1 and p40-2 partial digest peptides ranged in molecular weight from about 38,000 to 8,000. The electrophoretic migration of the individual peptides of intracellular p40-1 and p40-2 showed both similarities and differences. The differences in the peptide maps suggested that intracellular p40-1 and p40-2 differ in their primary amino acid sequence. Comparable results were obtained when partial proteolysis of intracellular p40-1 and p40-2 was carried out with chymotrypsin or trypsin (data not shown).

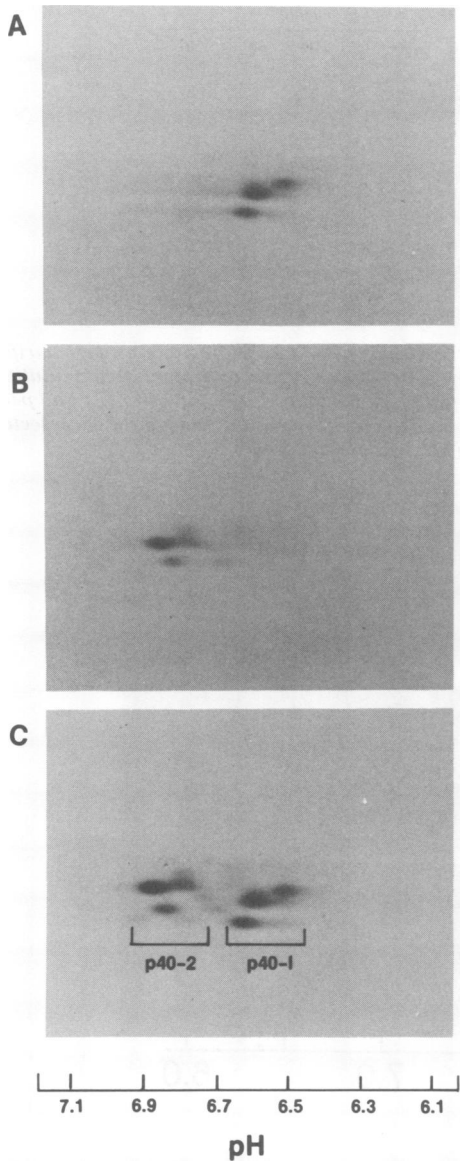

FIG. 2. *Two-dimensional gel electrophoresis of intracellular p40-1 and p40-2. [^{35}S]methionine-labeled, HSV-1- and HSV-2-infected cell extracts were preparatively immunoprecipitated and run on SDS-polyacrylamide gels. Autoradiographs of wet gels were obtained, and the intracellular p40 proteins were eluted as described in the text. Intracellular p40-1 and p40-2 proteins were separated by isoelectric focusing in the first dimension and by SDS-PAGE in the second dimension. Intracellular p40-1 proteins (A) and intracellular p40-2 proteins (B) were analyzed both separately and as a mixture (C).*

Two-dimensional gel analysis of the partial V8 protease digest peptides of intracellular p40-1 and p40-2. Partial digests of intracellular p40-1 and p40-2 were prepared by exposing the proteins to 50 μg of V8 protease per ml as described above. At this enzyme concentration, the peptide map of intracellular p40-1 is composed of six peptides with molecular weights of 20,000 (p20-1), 17,000 (p17-1), 16,000 (p16-1), 14,000 (p14-1), 11,000 (p11-1), and 8,000 (p8-1) (Fig. 3A). The p14-1 and p16-1 peptides were consistently seen as major peptides, whereas the p20-1, p17-1, p11-1, and p8-1 peptides were seen in variable proportions on fluorographs of SDS gels. Under identical conditions of digestion, intracellular p40-2 peptides having molecular weights of 23,000 (p23-2), 19,500 (p19.5-2), 14,000 (p14-2), 10,000 (p10-2), and 8,000 (p8-2) were observed (Fig. 3B). The peptides designated p23-2, p19.5-2, and p8-2 were not consistently seen in fluorographs of the intracellular p40-2 peptide profile after partial digestion.

Since the partial V8 protease digest products of intracellular p40-1 and p40-2 showed similarities and differences with regard to their electrophoretic mobility, it was of interest to further characterize these peptides by two-dimensional gel electrophoresis. The major intracellular p40 peptides were separated by charge in the first dimension in isoelectric focusing gels prepared by a modification of the method described by Breithaupt et al. (2) which allowed greater resolution in higher-pH regions. The intracellular p40-1 peptides resolved into three major spots that were identified by molecular weight as p14-1, p16-1, and p17-1 (Fig. 4A) which have isoelectric points of 8.2, 7.3, and 6.8, respectively. In contrast, the intracellular p40-2 peptides resolved into three major spots which were identified as p14-2, p19.5-2, and p23-2 with isoelectric points of 7.1, 6.8, and 6.3, respectively (Fig. 4B). As expected from these findings, each of the intracellular p40-1 and p40-2 peptides were separable when run as a mixture (Fig. 4C). The inability to detect additional peptide components may be the result of insufficient quantities for visualization or these peptides may not have focused in this gel system. Since each of the detectable intracellular p40-1 and p40-2 peptides had distinct isoelectric points and molecular weights, it was concluded that none of these peptides are identical.

Immunoprecipitation of intracellular p40-1 and intracellular p40-2 V8 protease cleavage products with guinea pig antiserum. Since it has been shown that intracellular p40-1 and p40-2 proteins contain type-specific and cross-reactive antigenic determinants (16), it was of interest to determine whether the individual peptides obtained by partial digestion of these protein classes contain distinct antigenic determinants. Intracellular p40-1 and p40-2 partial digest peptides, prepared as described above, were immunoprecipitated with several different

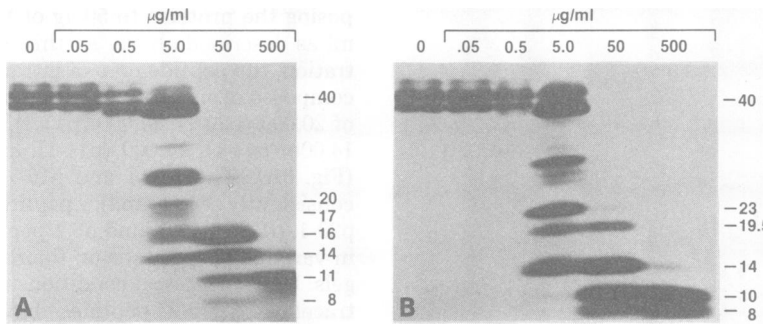

FIG. 3. *Peptide maps of intracellular p40-1 and p40-2. Intracellular p40-1 and p40-2 proteins were purified and digested with V8 protease as described in the text. Enzyme concentrations employed were 10-fold dilutions ranging from 500 to 0.05 μg/ml as indicated. The partial digest products of intracellular p40-1 (A) and p40-2 (B) were separated by SDS-PAGE, and a fluorograph was made. The numbers to the right of each section represent the approximate molecular weights (× 10^{-3}) of the polypeptides studied.*

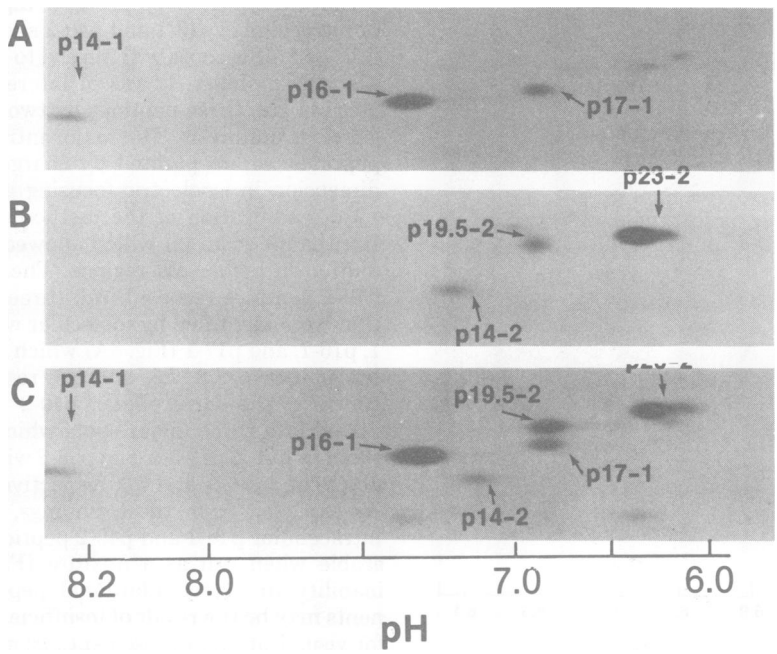

FIG. 4. *Two-dimensional gel electrophoresis of the partial digest peptides of intracellular p40-1 and p40-2. Purified intracellular p40-1 and p40-2 proteins were digested with 0.05 mg of S. aureus V8 protease per ml. [^{35}S]methionine containing intracellular p40-1 partial digest peptides (A) and intracellular p40-2 partial digest peptides (B) were analyzed separately and as a mixture (C). Peptides were separated by isoelectric focusing in the first dimension. Fluorographs were made after separation of peptides in the second dimension by SDS-PAGE.*

antisera from guinea pigs immunized with nucleocapsid p40-1, nucleocapsid p40-2, SDS-disrupted HSV-2 nucleocapsids, or a 30,000-dalton protein associated with simian herpesvirus SA8 (SA8 p30) intranuclear nucleocapsids. Immunoprecipitation studies with guinea pig antisera to nucleocapsid p40-1 and p40-2 indicated that SA8 p30 cross-reacts with nucleocapsid p40-1 and p40-2 (data not shown).

The partial digestion of intracellular p40-1 with V8 protease (50 μg) yielded five peptides (Fig. 5, lane A). Nonimmune guinea pig serum did not immunoprecipitate any of these peptides (Fig. 5, lane B). Two guinea pig antisera to nucleocapsid p40-1 consistently immunoprecipitated both the p16-1 and p14-1 peptides, whereas the immunoprecipitation of p17-1, p11-1, and p8-1 peptides was weak (Fig. 5, lanes C

and D). Guinea pig antisera against either nucleocapsid p40-2 (Fig. 5, lanes E and F) or HSV-2 nucleocapsids (Fig. 5, lane G), and guinea pig antiserum to SA8 p30 (Fig. 5, lane H) only immunoprecipitated the p14-1 peptide. These results indicate that the p16 peptide of p40-1 contains at least one type-specific antigenic determinant, whereas the p14 peptide of p40-1 contains one or more cross-reactive antigenic determinants.

Under the same conditions, partial digestion of intracellular p40-2 produced five peptides (Fig. 6, lane A). Nonimmune guinea pig serum did not precipitate any of these peptides (Fig. 6, lane B). Two guinea pig antisera against HSV-2 nucleocapsid p40 and the guinea pig antiserum against HSV-2 nucleocapsids consistently immunoprecipitated p23-2, p19.5-2, and p14-2, but showed various amounts of reactivity with the p10-2 and p8-2 peptides (Fig. 6, lanes C, D, and E). One guinea pig antiserum prepared against nucleocapsid p40-1 immunoprecipitated p14-2 and p23-2, but failed to immunoprecipitate the p19.5-2 peptide (Fig. 6, lane F). A second guinea pig antiserum to nucleocapsid p40-1 did not immunoprecipitate any of the HSV-2 p40 peptides (Fig. 6, lane G), but did precipitate homologous p40-1 peptides (Fig. 5, lane D). Guinea pig anti-SA8 nucleocapsid p30 antiserum immunoprecipitated p23-2 and p14-2 peptides (Fig. 6, lane H). Taken together, the data suggested that the p19.5-2 peptide contains at least one type-specific antigenic determinant, whereas p23-2 and p14-2 peptides contain cross-reactive determinants which may be antigenically identical or distinct. Nucleocapsid p40-1 also appears to contain at least one type-specific antigenic determinant which was detected on p14-1.

DISCUSSION

Intracellular p40-1 and p40-2 each contain three major protein components which appear

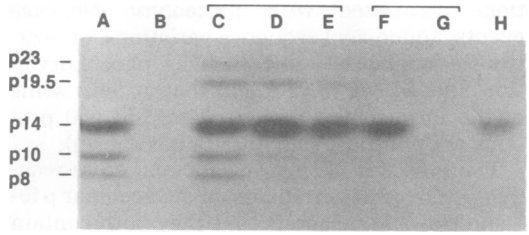

FIG. 6. *Immunoprecipitation of intracellular p40-2 partial digest peptides with guinea pig antiserum. Purified [^{35}S]methionine-labeled, intracellular p40-2 was partially digested with V8 protease and then reacted with guinea pig sera. The immune precipitates were separated by SDS-PAGE, and then fluorographs of these gels were prepared and analyzed on Kodak SB-5 X-ray film. Lanes: A, intracellular p40-2 partial digest peptides (not immunoprecipitated); B, peptides reacted with control nonimmune guinea pig serum; C and D, peptides immunoprecipitated with guinea pig antiserum against nucleocapsid p40-2; E, peptides immunoprecipitated with guinea pig antiserum against SDS-disrupted HSV-2 MS nucleocapsid; F and G, peptides immunoprecipitated with guinea pig antiserum against nucleocapsid p40-1; H, peptides immunoprecipitated with guinea pig antiserum against SA8 nucleocapsid p30. The antiserum used in lane G is the same antiserum used to immunoprecipitate intracellular p40-1 peptides shown in Fig. 5, lane D.*

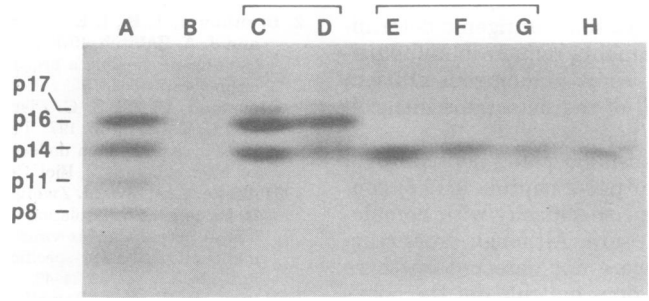

FIG. 5. *Fluorograph of intracellular p40-1 peptides immunoprecipitated with guinea pig antiserum. [^{35}S]-methionine-labeled, intracellular p40-1 was partially digested with V8 protease. Samples (10,000 cpm) of the protein digest were reacted with guinea pig control serum, guinea pig antisera prepared against nucleocapsid p40-1, p40-2, or SDS-disrupted HSV-2 nucleocapsids, and guinea pig antiserum to SA8 nucleocapsid p30. Lanes: A, intracellular p40-1 digest (not immunoprecipitated); B, peptides reacted with control nonimmune guinea pig serum; C and D, peptides precipitated with guinea pig antiserum against nucleocapsid p40-1; E and F, peptides immunoprecipitated with guinea pig antiserum against nucleocapsid p40-2; G, peptides immunoprecipitated with guinea pig antiserum against SDS-disrupted HSV-2 MS nucleocapsids; and H, peptides immunoprecipitated with guinea pig antiserum against SA8 nucleocapsid p30.*

to be immunologically related to one another and to the nucleocapsid p40. These soluble intracellular p40 protein components probably include precursor proteins which eventually are assembled into intranuclear nucleocapsids. Their heterogeneity in molecular weight and charge as seen by two-dimensional gel electrophoretic analysis may be the result of modifications associated with proteolytic cleavage events, amino acid sequence variations, or post-translational processing such as phosphorylation. Indeed, we have found that at least some of the intracellular and nucleocapsid p40 proteins are phosphorylated (data not shown).

The presence of type-specific and cross-reactive antigenic determinants on intracellular p40-1 and p40-2 (4) suggests that they both contain unique and common amino acid sequences. The two-dimensional gel pattern of proteins and V8 protease digest peptides also indicate the existence of unique amino acid sequences.

The immunoprecipitation of intracellular p40 V8 protease digest peptides by guinea pig antisera indicates that some peptides contain one or more type-specific antigenic determinants, whereas others contain at least one cross-reactive determinant. Although monoclonal antibody to nucleocapsid p40-1 and p40-2 immunoprecipitates the homologous intact purified protein, it did not precipitate any of the homologous or heterologous peptides after partial digestion with either V8 protease, trypsin, or chymotrypsin (data not shown). This may be because either (i) the appropriate antigenic determinant may be on a peptide not labeled with [^{35}S]methionine, (ii) the antigenic determinant may be cleaved by the enzyme, or (iii) the determinant may be conformational and altered after partial digestion. Although proteolytic cleavage of the protein probably destroys some antigenic determinants, other determinants remain immunologically reactive, since some homologous antisera are reactive with all of the detectable intracellular p40-1 or p40-2 peptides.

At least one intracellular p40-1 peptide (p16) and one intracellular p40-2 peptide (p19.5) consistently reacted type specifically with homologous guinea pig antisera. Although cross-reactive determinants were not detected on these peptides, this alone does not rule out the possibility of the presence of common amino acid sequences. Such sequences could be in a location which prevents interaction with antibody or in a structural position which normally renders them nonimmunogenic. The p14 peptides of intracellular p40-1 and p40-2 consistently showed strong cross-reactivity with anti-SA8 p30 antiserum and with all but one of the guinea pig antisera tested. This exception is indicative of a type-specific antigenic determinant(s) on the p14 peptide of intracellular p40-1, which suggests the presence of distinct amino acid sequences.

Recently, we have discovered that human antisera specifically immunoprecipitate intracellular p40 V8 protease peptides that contain type-specific antigenic determinants (Heilman et al., in preparation). As in the case of intracellular p40, the HSV-1 glycoprotein gD (gD-1) has also been shown to contain type-specific and cross-reactive antigenic determinants (10, 14). Since HSV-1 and HSV-2 are so closely related, it is likely that additional HSV proteins will be identified that contain both type-specific and cross-reactive antigenic determinants. Analysis of the antigenic determinants present on peptides derived from gD and perhaps other proteins, by the technique described here for intracellular p40, may be useful diagnostically or analytically to compare the gene products of HSV-1 and HSV-2 as well as other immunologically related herpesviruses (i.e., SA8, bovine mammalitis virus, B virus). Rapid advances in the production of monoclonal antibodies against herpesvirus proteins (10, 17; Showalter et al., in press) should facilitate the purification of herpesvirus proteins and allow similar structural and immunological studies to be carried out.

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