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Structural Analysis of the Capsid Polypeptides of Herpes Simplex Virus Types 1 and 2

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Capsids of herpes simplex virus (HSV) types 1 and 2 contain seven polypeptides ranging in molecular weight from 154,000 to 12,000 (termed NC-1 through NC-7 in order of descending molecular weight). Antibodies prepared to HSV-1 capsid polypeptides isolated from sodium dodecyl sulfate-polyacrylamide gels reacted in an immunofluorescence assay against HSV-1-infected KB cells. Three of the antibodies (anti-NC-1, anti-NC-2, and anti-NC-3,4) also reacted with HSV-2-infected cells. Tryptic peptide analysis showed that each of the HSV-1 capsid polypeptides had a unique methionine peptide profile, and none appeared to be derived from the major capsid polypeptide. Comparative peptide analysis of HSV-1 and HSV-2 showed that one polypeptide (NC-7, 12,000 molecular weight) had an identical methionine peptide profile and a very similar arginine peptide profile in both virus types. The arginine peptide profile of NC-7 of HSV-1 was very different from the arginine profile of KB histone H4. Although there were certain intertypic similarities in the methionine peptide profiles of the other capsid components, especially in NC-1 (the major capsid protein), there was no case where the tryptic peptides were identical in the two virus types.

Gibson and Roizman (14, 15) analyzed the protein composition of herpes simplex virus type 1 (HSV-1) and HSV-2 nucleocapsids. Two major intranuclear forms were described: (i) an A form lacking DNA and containing one minor and three major structural polypeptides; and (ii) a B form containing all of the polypeptides found in the A capsid as well as two additional polypeptides. Recently we presented a preliminary report (G. H. Cohen, W. C. Lawrence, M. Ponce de Leon, S. Vernon, and H. Diggelmann, Abstr. Annu. Meet. Am. Soc. Microbiol. 1978, S42, p. 219) confirming these results (14, 15) and describing an additional 12,000-molecular-weight polypeptide (NC-7). Heilman et al. (16) also found a protein pattern for capsids similar to that reported by Gibson and Roizman (14, 15) and confirmed our report that HSV capsids contain a 12,000-molecular-weight polypeptide.

Although HSV-1 differs significantly from HSV-2 in a number of biological, biochemical, and biophysical characteristics (29), the two forms also exhibit similarities. DNA-DNA hybridization studies indicate that HSV-1 and HSV-2 exhibit approximately 50% homology in their DNA with 85% matching of base pairs of the homologous regions (18). HSV-1 and HSV-2 capsid polypeptides have very similar molecular weights, and the possibility exists that they have the same or similar amino acid sequences. However, the major capsid protein of HSV-1 (154,000 molecular weight) has a slightly faster electrophoretic mobility than the corresponding protein found in HSV-2 capsids (155,000 molecular weight) (3, 8). This difference was utilized to determine the location of the gene coding for the (NC-1) polypeptide in intertypic recombinants (20, 22). Even though the major capsid polypeptides of HSV-1 and HSV-2 are distinguishable on the basis of electrophoretic mobility, they are related immunologically (8, 16, 26). In addition, Heilman et al. (16) showed that a second capsid polypeptide, p40 (equal to NC-3), found in the heavy or B capsids of HSV-1 and HSV-2, had both cross-reactive and type-specific antigenic determinants. Except for reports of amino acid composition (9) and of disulfide linkages in HSV-2 polypeptides (38), little is known about the chemical structure of the HSV capsid or its polypeptides.

The purpose of the present studies was to characterize the capsid polypeptides of HSV-1 and HSV-2. We will present evidence to show that all of the HSV-1 capsid polypeptides isolated from sodium dodecyl sulfate (SDS)-polyacrylamide gels are immunogenic and stimulate

production of antibodies that react with HSV-1-infected cells. Three of these also react with HSV-2-infected cells. Tryptic peptide analysis showed that each of the HSV-1 capsid polypeptides contained a unique set of methionine peptides, and none appeared to be derived from the major (NC-1) capsid polypeptide. Comparative analysis showed that two polypeptides, NC-1 and NC-7, had similar tryptic peptide profiles in HSV-1 and HSV-2. The other capsid components of HSV-1 showed fewer structural similarities with the corresponding components of HSV-2.

MATERIALS AND METHODS

Cell cultures. Conditions for the growth and maintenance of KB cells and baby hamster kidney cells have been described previously (7).

Virus preparation and titration. The procedures used for the preparation of virus stocks of HSV-1 (strain HF) and HSV-2 (Savage strain) have been described previously (5, 7).

Preparation of infected KB cells for nucleocapsid purification. KB cells were propagated in plastic roller bottles (490 cm^2) essentially as suggested by C. Shipman, Jr. (personal communication). The roller bottles were pretreated with Eagle minimal essential medium supplemented with 10% calf serum for 1 h at 36°C, and then were seeded with 2.5×10^7 KB cells. Monolayers were obtained within 72 h and were infected with 0.5 PFU of HSV-1 or 10 PFU of HSV-2 per cell. At 5 h postinfection, the medium was replaced with 50 ml of Eagle minimal essential medium containing 1/10 of the usual amount of methionine and 5 μCi of [^{35}S]methionine (specific activity, >400 Ci/mmol), or 5 μCi of L-[*methyl*-^{3}H]methionine (specific activity, 100 Ci/mmol), or 1 μCi of [^{14}C]arginine (specific activity, 270 mCi/mmol), or 5 μCi of [^{3}H]arginine (specific activity, 18 Ci/mmol) per ml. All radioisotopes were purchased from New England Nuclear Corp. The cells were incubated for 24 h at 34°C, harvested by centrifugation, and washed with 0.01 M phosphate buffer (pH 7.2).

Purification of nucleocapsids. The method employed was a composite of four established procedures (9, 14, 23, 28). Briefly, nuclei were isolated from infected cells (14) and suspended in phosphate buffer to a density of 10^6 nuclei per ml. The suspension was homogenized and sonicated three times for 30 s. Sodium deoxycholate was added to a final concentration of 0.5%, and the suspension was incubated for 1 h at 36°C with constant agitation. DNase (100 μg/ml) and RNase (120 μg/ml) were added to the preparation in the presence of 1 mM $MgCl_2$ and incubated at 36°C for 1 h. $MgCl_2$ (final concentration, 0.02 M) was then added to precipitate the deoxycholate. The precipitate was removed by centrifugation at 10,000 × *g* for 20 min, and the supernatant fluid was pelleted through 36% sucrose in an SW27 rotor at 90,000 × *g* for 90 min. The pellet was suspended in 5 ml of 40% (wt/vol) CsCl, sonicated briefly, overlaid with a 12 to 30% linear CsCl gradient (12 ml), and centrifuged in an SW27.1 rotor at 40,000 × *g* for 17 h. The capsids of HSV-1 were visible as two bands in the center of the gradient. HSV-2 capsids were not resolved into two populations, but appeared as a broad band. When required, further purification of the heavy (buoyant density = 1.2890 g/cm^3) or light (buoyant density = 1.2750 g/cm^3) HSV-1 capsids was achieved by a second sedimentation step through a linear 22 to 40% CsCl gradient for 90 min at 90,000 × *g*. Capsids stored in 38% CsCl at 4°C were stable for several weeks. The purity of the preparations was determined in three ways: (i) by electron microscopy (35, 36); (ii) by SDS-polyacrylamide gel electrophoresis (PAGE) analysis; and (iii) by mixing experiments in which [^{35}S]methionine-labeled uninfected nuclei were added to unlabeled purified capsids before a second round of purification. The purified capsid preparation contained less than 5% of the label of the mixed starting preparation, and SDS-PAGE analysis revealed no radioactive bands comigrating with capsid polypeptides.

Histones were extracted from uninfected KB cell nuclei according to the procedure of Bonner et al. (1).

SDS-PAGE analysis. SDS-PAGE was carried out in slabs of 10% acrylamide cross-linked with 0.4% *N,N*-diallyltartardiamide or on slabs of 18% acrylamide cross-linked with 0.26% *N,N*-methylenebisacrylamide as described previously (6, 10). After electrophoresis, the gels were stained with Coomassie brilliant blue, dried on filter paper, and placed in contact with Kodak X-Omat R (XR-5) film. For fluorography, the gels were impregnated with 2,5-diphenyloxazole (2) before drying, and exposed to X-ray film at −70°C. Protein standards ranging from 15,000 to 130,000 daltons were run on each gel (6).

Preparation of samples for tryptic peptide analysis. Polypeptides were eluted from *N,N*-methylenebisacrylamide cross-linked gels and prepared for trypsinization according to the procedure of Vogt et al. (37). *N,N*-Diallyltartardiamide cross-linked gel slices were dissolved in 2% periodic acid, according to the procedure of Gibson (13), and prepared for trypsinization as described previously (10). Both methods gave identical tryptic methionine peptide profiles for the major capsid polypeptides. Trypsinization and ion-exchange chromatography on Chromobeads P (Technicon) were carried out according to the procedure described by Vogt (37) and modified as described previously (10).

Immunological procedures. (i) Preparation of antisera. Antisera to each of the capsid polypeptides of HSV-1 were prepared in the following manner. Capsid polypeptides prepared from unlabeled cells were located in unstained gels by bracketing with labeled polypeptides. After electrophoresis, the gels were washed with water, covered with plastic wrap, and placed in contact with X-ray film at 4°C. The gel slices containing the polypeptides were macerated and mixed with Freund complete adjuvant. Approximately 538 μg of capsid protein was subjected to SDS-PAGE. Rabbits received four weekly intramuscular injections followed by a 10-day rest period. Each rabbit then received a subcutaneous injection and was bled 9 days later. We estimate that the total amount of protein used for immunization was as follows: NC-1, 324 μg; NC-2, 54 μg; NC-3,4, 64 μg; NC-5, 59 μg; NC-7, 37 μg. Immunoglobulin G was prepared from each antiserum

as described by Montgomery et al. (21).

(ii) Immunofluorescence. A modification of the indirect procedure described by Reed et al. (27) was used. Briefly, monolayers of KB cells were grown on Lab-Tek slides (Miles Laboratories, Inc.) infected with HSV-1 or HSV-2 and fixed with 3.7% formaldehyde at 14 h postinfection. The slides were washed with phosphate-buffered saline, dehydrated for 7 min at −20°C with acetone, and washed with phosphate-buffered saline. The chambers were overlaid for 30 min at 36°C with 50 μl of phosphate-buffered saline containing the appropriate antibody. The slides were washed three times with phosphate-buffered saline and incubated with a mixture of fluorescein isothiocyanate-conjugated goat anti-rabbit immunoglobulin G and rhodamine-conjugated bovine serum albumin. The latter component allowed us to visualize cells that did not specifically react with antibody.

RESULTS

Analysis of HSV-1 and HSV-2 capsids by SDS-PAGE. Figure 1 shows a fluorogram of electrophoretically separated HSV-1 and HSV-2 capsid polypeptides labeled with radioactive methionine. HSV-1 capsids (track 1) consisted of seven polypeptide bands with molecular weights of 154,000 (154K) (NC-1), 50K (NC-2), 40K (NC-3), 38K (NC-4), 33K (NC-5), 26K (NC-6), and 12K (NC-7). HSV-2 capsids (track 2) also consisted of seven bands, and with three exceptions their molecular weights were roughly comparable to the corresponding polypeptides seen in HSV-1. These have also been designated NC-1 through NC-7 in order of decreasing molecular weight. The exceptions were NC-1 and NC-3,4. Figure 1 shows that NC-3 of HSV-1 had the same molecular weight (40K) as NC-4 of HSV-2. NC-1 of HSV-2 was slightly larger than NC-1 of HSV-1, although the difference is not evident in Fig. 1. NC-6 in HSV-1 does not show up well in Fig. 1. This component was present in such low quantities that it was not studied any further.

Our present studies employed isopycnic CsCl centrifugation for capsid purification. A light-capsid population usually represented 90% and a heavy-capsid population comprised 10% of the total recoverable radioactivity. In agreement with previous studies (14–16), we found that these two populations differed not only in density but in two other respects: (i) electron microscopic studies showed that, whereas heavy capsids appeared to be impermeable to negative stain, 80% of the light capsids usually were permeable; (ii) SDS-PAGE analysis indicated that polypeptides NC-3 and NC-4 were underrepresented in light capsids. In our hands, HSV-2 capsids could not be resolved into two populations.

To investigate whether the capsid polypeptides of HSV-1 and HSV-2 exhibited immunological cross-reactivity, antibodies were prepared to individual gel bands of HSV-1 and utilized in an immunofluorescent assay.

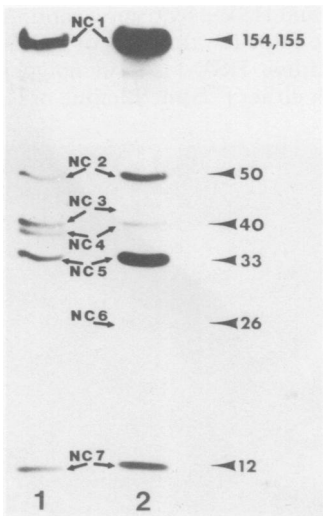

FIG. 1. *SDS-PAGE analysis of purified HSV-1 and HSV-2 capsids. Fluorogram of a 10% N,N-diallyltartardiamide gel. Track 1, [^{3}H]methionine-labeled HSV-1 capsids; track 2, [^{35}S]methionine-labeled HSV-2 capsids. Molecular weights (×10^3) on the right.*

Immunological studies of HSV-1 and HSV-2 capsids. Figure 2 shows examples of the type of immunofluorescence observed when antibody prepared to each of the HSV-1 capsid polypeptides was reacted with HSV-1-infected KB cells. Antibody prepared against NC-1, NC-2, and NC-3,4 (the two closely migrating bands were evaluated as a mixture) exhibited strong nuclear reactions. Antibody against the lower-molecular-weight capsid polypeptides (NC-5 and NC-7) showed much weaker but still positive reactions.

Table 1 summarizes our observations of the relative intensity and location of fluorescence seen when these antibodies were reacted with HSV-1- and HSV-2-infected cells. Anti-NC-1, -NC-2, and -NC-3,4 reacted against cells infected with either virus type. In each case the homologous reaction was stronger than the heterologous reaction. These results agree with those reported for NC-1 (8, 16, 26) and NC-3 (16). Anti-NC-5 and anti-NC-7 reacted weakly with HSV-1-infected cells and showed no detectable cross-reactivity with HSV-2-infected cells at the antibody levels employed (Table 1). Because the correlation of immunological cross-reactivity with structural similarity is difficult to predict (17, 20, 23), we next examined the polypeptides

of HSV-1 and HSV-2 by tryptic peptide analysis.

Tryptic peptide analysis of HSV-1 capsid polypeptides. HSV-1 capsid polypeptides labeled with either [^{35}S]methionine or [^{3}H]methionine were separated by SDS-PAGE. Each component was extracted from the gel, trypsinized, and cochromatographed on a column of Chromobeads P with the tryptic peptides of the major capsid polypeptide NC-1. Figure 3 shows that the tryptic digest of NC-1 could be resolved into approximately 14 methionine peptides in addition to the flow-through fraction. Free methionine eluted at fraction 38, a position that did not correspond to any of the major methionine peptides of NC-1. The tryptic peptides of NC-2 (Fig. 4A) were resolved into a complex pattern that was quite different from that of NC-1. Two methionine peptides (at fractions 130 and 155) clearly overlapped, suggesting that these two peptides might be structurally similar. However, it is also possible that the overlapping peaks in this complex pattern represented structurally different peptides with similar elution properties (12). The tryptic peptide profile of NC-3,4 (Fig. 4B) was different from that of NC-1 or NC-2. The profile of NC-5 was strikingly different from that of any of the other capsid polypeptides and was notable in that: (i) all of the peptides eluted at acidic pH values, and (ii) one peptide exhibited some variability in elution. The arrow in Fig. 4C indicates the position of this peak, which was seen prominently in some experiments and was a minor peak in other experiments. Finally,

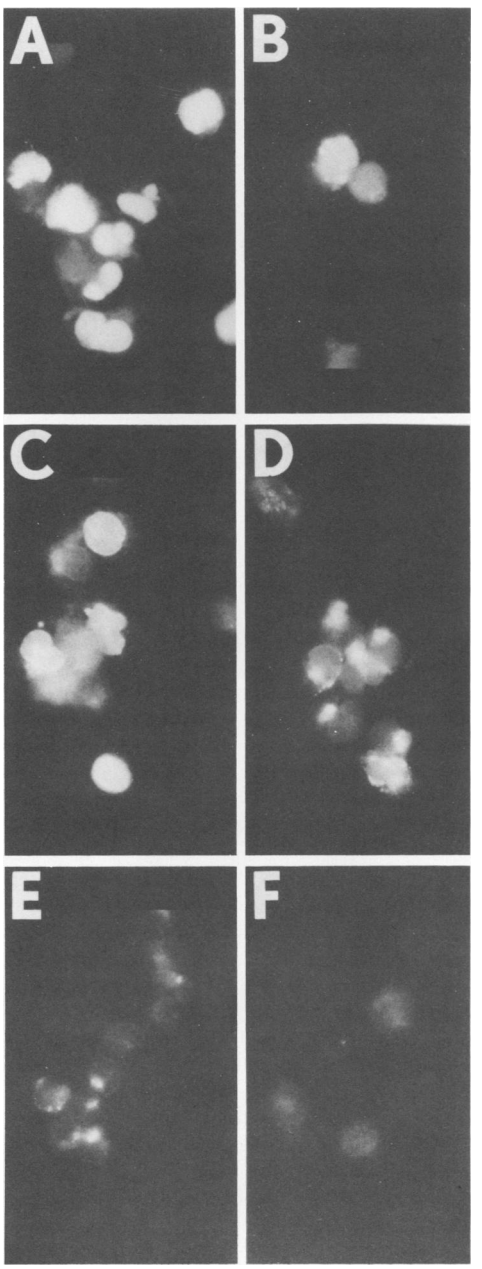

FIG. 2. *Immunofluorescence analysis of HSV-1-infected KB cells using antibodies to the capsid proteins. The concentrations of antibodies used in this assay are given in Table 1. (A) Anti-NC-1; (B) anti-NC-2; (C) anti-NC-3,4; (D) anti-NC-5; (E) anti-NC-7; (F) normal rabbit antibody, 1.3 mg/ml.*

TABLE 1. *Summary of immunofluorescence analysis of HSV-1- and HSV-2-infected cells using antibodies to HSV-1 capsid proteins*

Antiserum prepared to HSV-1 capsid component:	Cells infected with HSV type	Concn of antibody[a] (mg/ml)	Relative immunofluorescence intensity[b]	
			Nucleus	Cytoplasm
NC-1	1	0.06	+++	++
	2	0.6	+++	+
NC-2	1	0.06	+++	−
	2	1.2	++	−
NC-3,4[c]	1	0.18	+++	+
	2	1.8	++	−
NC-5	1	0.68	++	−
	2	1.7	−	−
NC-7	1	0.44	+	−
	2	1.6	−	−

[a] The concentration of antibody (immunoglobulin G) utilized against HSV-1-infected cells is the lowest concentration yielding maximum fluorescence. For HSV-2, where the reaction was positive, the concentration of antibody is the lowest amount yielding maximum fluorescence. When the reaction was negative (NC-5 and NC-7 versus HSV-2), the concentration shown in the table was the highest tested.

[b] Strongest reaction indicated by +++; negative reaction indicated by −.

[c] This antiserum was prepared against a mixture of NC-3 and NC-4.

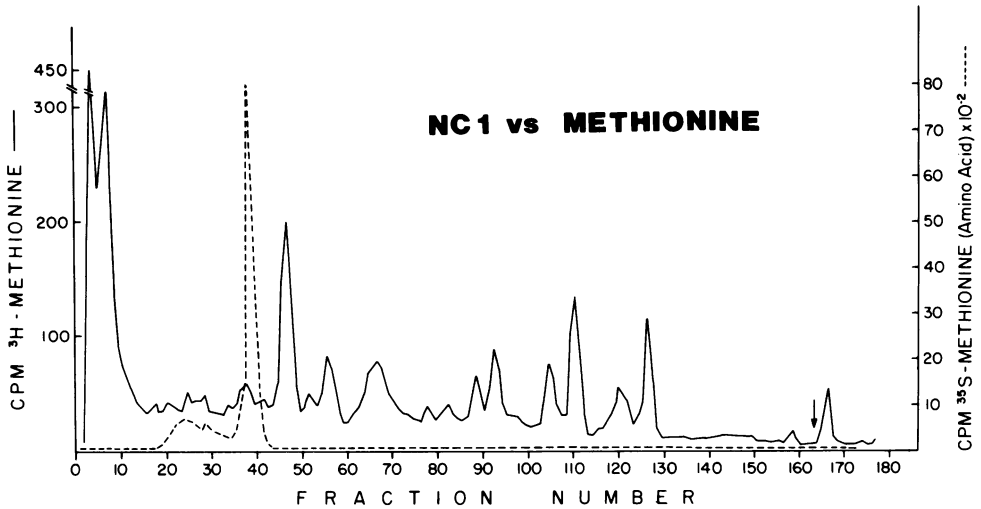

FIG. 3. *Tryptic peptide analysis of methionine-labeled NC-1 obtained from HSV-1 capsids. NC-1 labeled with [³H]methionine was co-chromatographed on a column of Chromobeads P with [³⁵S]methionine. In this and each of the following figures, the arrow represents the start of a basic wash of the column.*

the tryptic peptide pattern for NC-7 (Fig. 4D) was rather simple, consisting of one resolved methionine peptide which eluted toward the basic end of the pH gradient. This peptide did not correspond to that of free methionine (cf. Fig. 3), nor did it appear to correspond to a methionine peptide of any of the other capsid polypeptides. Thus NC-7 does not appear to be a breakdown product of a larger capsid protein.

This series of experiments demonstrated that each of the HSV-1 capsid polypeptides appeared to contain a characteristic and unique set of tryptic peptides. In one case (NC-2) there were two peptides that might contain sequences similar to those in NC-1. However, none of the other capsid polypeptides contained tryptic peptides that coeluted with those of NC-1, and none appeared to be similar to any other capsid polypeptide.

Comparison of the tryptic methionine peptides obtained from HSV-1 and HSV-2 capsid polypeptides. In these experiments the tryptic peptides of each HSV-1 capsid polypeptide were cochromatographed with the tryptic peptides of the corresponding polypeptide from HSV-2. If the high degree of intertypic DNA homology (18) were reflected by similar amino acid sequences in the capsid polypeptides, we would expect to see overlaps (complete or partial) in the peptide profiles. Figure 5A shows that the overall elution profiles for NC-1 of HSV-1 and HSV-2, though not identical, appeared quite similar, and several peaks appeared to overlap. The second case where there was a strong similarity is shown in Fig. 5B. The methionine profile of NC-7 from HSV-1 was essentially the same as that of NC-7 from HSV-2. However, the simplicity of the methionine peptide profile for NC-7 made it difficult to conclude that this protein is identical in the two virus types. Figure 6 shows that the arginine peptide profile for NC-7 was very similar but not identical in HSV-1 and HSV-2. The data in Fig. 5 and 6 suggest that there may be a high degree of intertypic homology of amino acid sequence in these two polypeptides.

Figure 7 shows the elution profiles for three capsid polypeptides of HSV-1 and HSV-2 that exhibited fewer overlapping peptides. The resolved methionine peptides of NC-2 isolated from HSV-1 and HSV-2 (Fig. 7A) were clearly different, even though the polypeptides had the same apparent molecular weight by SDS-PAGE and cross-reacted immunologically. Parenthetically, the NC-2s of HSV-1 and HSV-2 contain a significant amount of radioactive label which elutes at a basic pH. The methionine peptides of the NC-3,4 region (Fig. 7B) of HSV-1 and HSV-2 were different, despite intertypic immunological cross-reactivity. However, in one region of the elution profile (fractions 40 to 80), the patterns looked similar, and it is possible that the common antigenic site(s) might be found in this region. Figure 7C shows that NC-5 of HSV-1 had two methionine peptides that coeluted with peptides of NC-5 of HSV-2. In this case, the possibility for structural similarity shown by peptide analysis was not consistent with the negative immunological data. In summary, Fig. 7 demonstrates that there are impor-

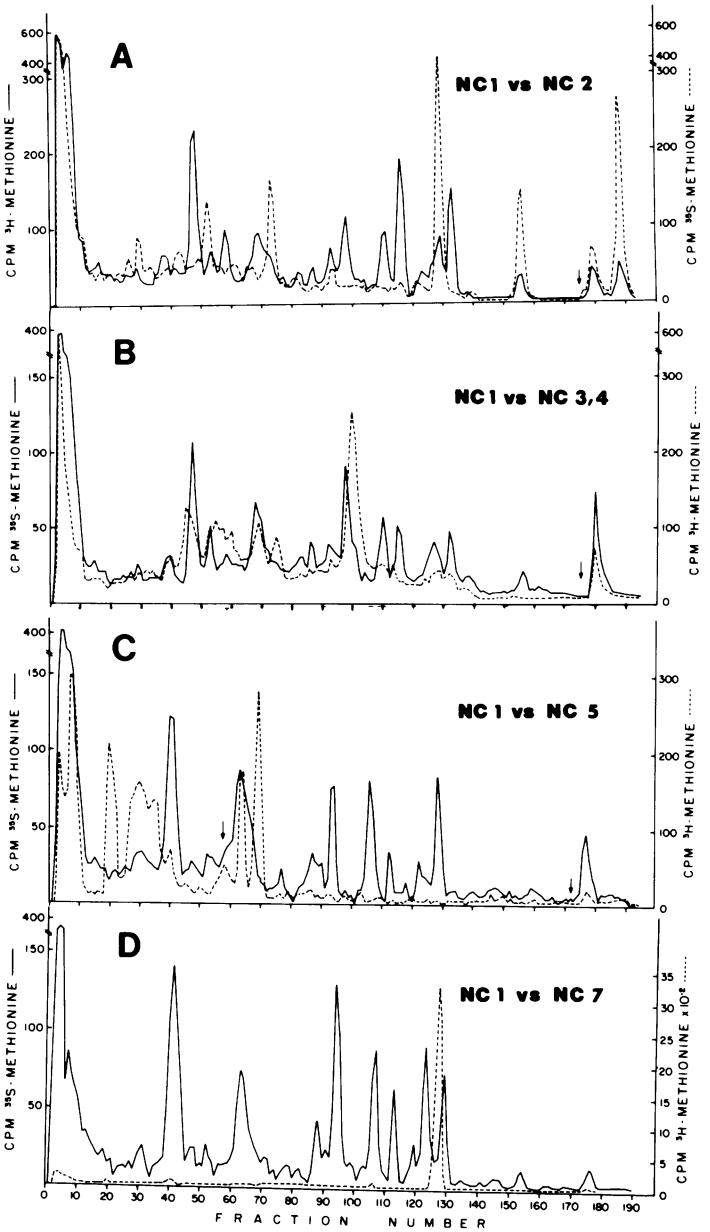

FIG. 4. *Tryptic peptide analysis of methionine-labeled HSV-1 capsid polypeptides. Tryptic peptides of NC-1 (——) labeled with either [^{35}S]methionine or [^{3}H]methionine were cochromatographed on a Chromobeads P column with [^{3}H]methionine- or [^{35}S]methionine-labeled peptides (----) of: (A) NC-2, (B) NC-3,4, (C) NC-5, or (D) NC-7. In each case the left-hand ordinate represents NC-1. The arrow in (C) (fraction 58) marks the position of the variable peptide.*

tant structural differences between polypeptides with similar molecular weights, and Fig. 5, 6, and 7 demonstrate that structural similarities or differences may not be consistent with immunological data.

Is NC-7 the host cell histone H4? The NC-7s of HSV-1 and HSV-2 had identical methionine tryptic peptides and very similar arginine peptide profiles. Each of these components might be a host protein or a highly conserved viral component. Figure 8 shows that NC-7 of HSV-1 migrated in SDS-PAGE gels with a mo-

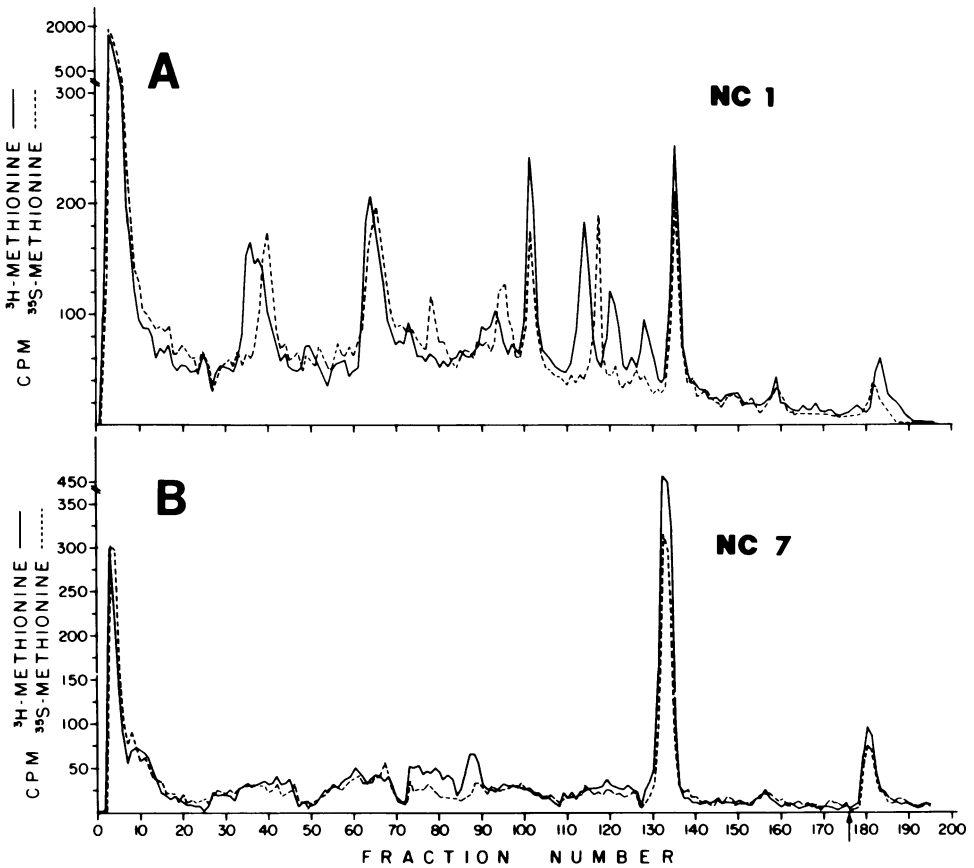

FIG. 5. *Comparative tryptic peptide analysis of NC-1 and NC-7 isolated from HSV-1 and HSV-2 capsids. Cochromatography of: (A) [³H]methionine-labeled NC-1 from HSV-1 (——) with [³⁵S]methionine-labeled NC-1 from HSV-2 (---); (B) [³⁵S]methionine-labeled NC-7 from HSV-1 (——) with [³⁵S]methionine-labeled NC-7 from HSV-2 (---).*

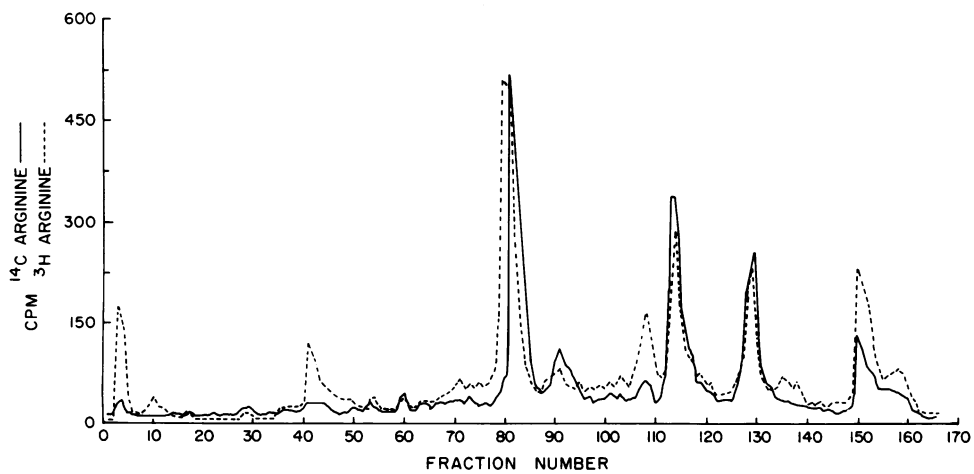

FIG. 6. *Comparative tryptic peptide analysis of NC-7 from HSV-1 and HSV-2 capsids. Cochromatography of [¹⁴C]arginine-labeled peptides from HSV-2 (——) with [³H]arginine-labeled peptides from HSV-1 (---).*

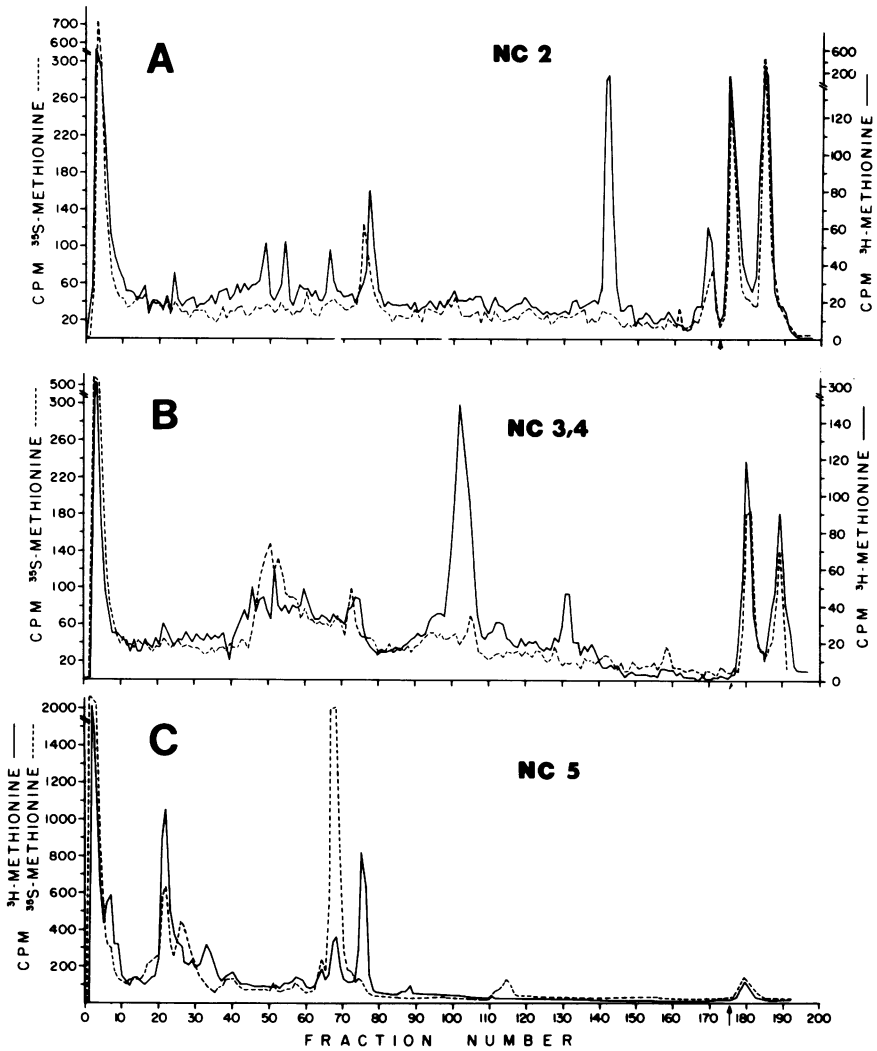

FIG. 7. *Comparative tryptic peptide analysis of NC-2, NC-3,4, and NC-5 from HSV-1 and HSV-2 capsids. Cochromatography of (A) [^{3}H]methionine-labeled NC-2 from HSV-1 (——) with [^{35}S]methionine-labeled NC-2 from HSV-2 (---); (B) [^{3}H]methionine-labeled NC-3,4 from HSV-1 (——) with [^{35}S]methionine-labeled NC-3,4 from HSV-2 (---); (C) [^{3}H]methionine-labeled NC-5 from HSV-1 (——) with [^{35}S]methionine-labeled NC-5 from HSV-2 (---).*

bility similar to that of H4 histone of uninfected KB cells. To compare NC-7 with H4 we decided to examine the arginine tryptic peptides of these two polypeptides. KB cells were labeled with [^{3}H]arginine for 18 h, a nuclear fraction was prepared, and the histone fraction was isolated (1). HSV-1 capsids were prepared from [^{14}C]-arginine-labeled KB cells. These preparations were subjected to SDS-PAGE, and the bands corresponding to KB-H4 and NC-7 were extracted from the gel, oxidized, trypsinized, and cochromatographed on Chromobeads P. Figure 9 shows that the tryptic peptide profiles for KB-H4 and NC-7 are different. From this experiment we conclude that NC-7 is not the KB host histone H4.

DISCUSSION

This study was designed to examine immunological and structural properties of the capsid polypeptides of HSV-1 and HSV-2. We found that each of the HSV-1 capsid polypeptides extracted from SDS gels was capable of inducing antibody which reacted with HSV-1-infected cells. In addition, antibody to the HSV-1 capsid polypeptides NC-1, NC-2, and NC-3,4 also re-

acted with HSV-2-infected cells. Similar results have been reported previously for NC-1 (8, 16) and NC-3 (16). Since the antibodies were prepared to SDS-denatured proteins, it is possible that sites not normally available to provoke an immune response were exposed and became immunogenic. Thus, as was shown in the case of simian virus 40 and polyoma virus (32) as well as for adeno-associated virions (17), the sera we prepared may turn out to have greater cross-reactivity than sera prepared against undenatured polypeptides. The immunological cross-reactivity that we observed reflects intertypic structural similarities, but the extent of these similarities is difficult to assess. It is possible that structural homology between polypeptides may actually be great even though the sites responsible for immunological cross-reactivity are hidden in the native protein. For example, α-fetoprotein and serum albumin have a marked structural similarity but do not cross-react unless antibody prepared against unfolded polypeptide chains is utilized (30).

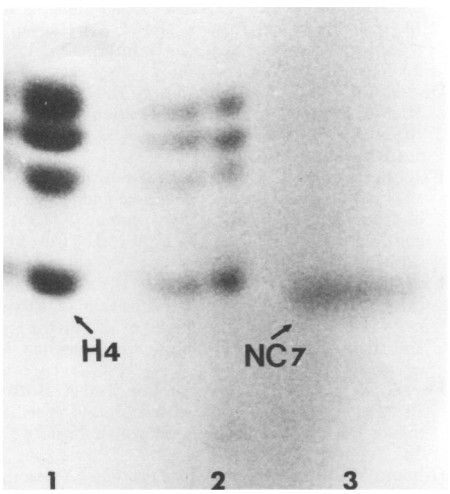

FIG. 8. *SDS-PAGE analysis of NC-7 from HSV-1 and KB cell histones. Fluorogram of an 18% N,N-methylenebisacrylamide-linked gel. Only the lower-molecular-weight region of the gel is shown. Track 1, [^{3}H]arginine-labeled histones extracted from uninfected KB cells; track 2, same as track 1, half as much protein; track 3, [^{14}C]arginine-labeled NC-7 from HSV-1.*

Tryptic peptide analysis was utilized to establish two points: (i) whether each of the HSV-1 capsid polypeptides is composed of a unique set of methionine-labeled peptides; and (ii) the degree of structural relatedness between corresponding HSV-1 and HSV-2 capsid polypeptides. Our data indicate that the capsid components observed on SDS-PAGE are not generated as a result of proteolytic cleavage of the major capsid polypeptide (NC-1), and each appears to consist of a unique set of methionine peptides. In one instance (NC-1 and NC-2 of HSV-1, Fig. 4A), several methionine peptides are shared. It is important to bear in mind that because the methionine peptide patterns are complex, it is possible that there was random overlapping of structurally different peptides (12). Further studies will be required to determine whether these peptides are truly shared among viral structural components. The degree of relatedness between the corresponding HSV-1 and HSV-2 capsid polypeptides is more difficult to assess. It is clear that in all cases except one (NC-7), the intertypic methionine peptide profiles were not identical in spite of the fact that the proteins have similar apparent molecular weight values. In the case of NC-7, small differences in the arginine peptide profiles were detected. Thus, we can conclude that there is no complete intertypic conservation of amino acid sequence in proteins that presumably have similar structural functions.

One explanation for the differences in the tryptic peptide patterns for HSV-1 and HSV-2 capsid polypeptides is that they are due to intratypic variability. Such variability has been demonstrated for certain HSV-1 virion polypeptides

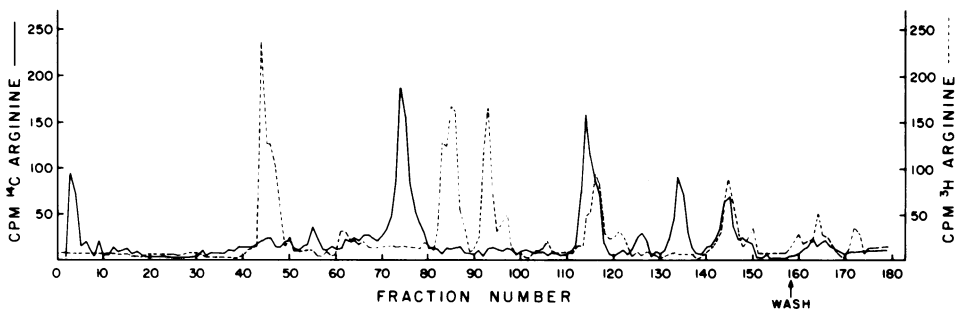

FIG. 9. *Comparative tryptic peptide analysis of NC-7 from HSV-1 and KB cell histone H4. Cochromatography of [^{14}C]arginine-labeled peptides from NC-7 (——) with [^{3}H]arginine-labeled peptides from H4 (---).*

(24) but not for all components (25). Since we examined only one strain of HSV-1 and HSV-2, we cannot conclude that no intratypic variability exists. Some of the differences we observed may be due to this phenomenon rather than to intertypic differences. Assuming, however, that the differences we observed in the tryptic peptide profiles are due to intertypic rather than intratypic variability, tryptic peptide analysis could be utilized to verify the parental origin of polypeptides in HSV-1 × HSV-2 recombinants. It is interesting that the intertypic forms of NC-1 appear to contain a significant number of peptides in common. Furthermore, the arginine and methionine peptides of NC-7 are closely conserved between the two virus types. We speculate that some of these peptides contain highly conserved amino acid sequences.

HSV-specific polypeptides smaller than 25K have been described (19, 28), but little is known about their structure or functional properties. Low-molecular-weight polypeptides isolated from other viruses such as the papovaviruses and adenovirus apparently are important components in the condensation of viral DNA. Papovaviruses, for example, utilize host histones for this purpose (31), whereas adenovirus core proteins are basic and presumably function in a similar manner (11). We have shown here by tryptic peptide analysis that NC-7 is not the KB H4 histone. Y. Roth (submitted for publication) has presented data that NC-7 could be extracted with acid from infected cell nuclei, chromatin of HSV-1-infected cells, or purified HSV-1 capsids. Low-molecular-weight basic proteins have already been reported to be present in herpesviruses (4, 34), and it is possible that NC-7 is a basic capsid protein involved in the events leading to the packaging of HSV DNA.

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