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NOTES

Comparison of the Virion Proteins Specified by Herpes Simplex Virus Types 1 and 2

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Purified herpes simplex virus type 2 (HSV-2) virions were found to contain approximately the same number of polypeptides as HSV type 1 (HSV-1) virions. Comparisons of the structural proteins specified by five independent HSV-2 isolates revealed some minor differences in their electrophoretic profiles on sodium dodecyl sulfate-acrylamide gels; certain invariant features of the electrophoretic profiles, however, allowed clear differentiation between all the HSV-2 isolates and HSV-1.

Isolates of herpes simplex virus (HSV) have been classified into two groups, designated HSV-1 and HSV-2, on the basis of antigenic differences detected in neutralization assays (11, 12, 16). The two antigenic types exhibit a number of biological differences (3-5, 13), and molecular hybridization studies have revealed that the DNAs from one HSV-1 isolate and one HSV-2 isolate share only 50% of their sequences in common (10), suggesting considerable genetic divergence between the two types.

Inasmuch as herpesvirion glycoproteins are probably the targets of neutralizing antibodies (2, 9, 14; P. G. Spear, *in Proceedings of the Second International Symposium on Herpesviruses and Oncogenesis*, in press), it is reasonable to suppose that the antigenic differences detected by these antibodies reflect differences in the structures of envelope proteins specified by HSV-1 and HSV-2. Moreover, Honess et al. (8) presented evidence that some of the antigenic determinants detected on HSV-1 nucleocapsids may not be present on HSV-2 nucleocapsids. Direct comparisons of HSV-1 and HSV-2 structural proteins have been hampered, however, by difficulties encountered in purifying HSV-2 virions. We report here the successful purification of virions from five independent HSV-2 isolates along with comparisons of their polypeptides and HSV-1 virion polypeptides by sodium dodecyl sulfate (SDS)-acrylamide gel electrophoresis. These comparisons revealed that most of the virion proteins specified by HSV-2 had electrophoretically identical counterparts in all the HSV-2 isolates; certain features of the electrophoretic profiles, however, clearly differentiated the HSV-2 isolates from those of HSV-1.

Three of the HSV-2 isolates used in this study (G, H445, and 67GN) were recovered from genital lesions and two (118 and 147) were recovered from lesions on the thigh; all five were classified as HSV-2 on the basis of neutralization assays. Strain H445, provided by A. Nahmias (Emory University, Atlanta, Ga.), was isolated from the cervix of a woman who subsequently developed cervical carcinoma; strain 67GN, provided by P. Balduzzi (University of Rochester, Rochester, N. Y.), was isolated from the vagina of a woman with mild dysplasia of the cervix. Several published reports provide additional information about G (4, 10, 15), 118 (19), and 147 (1) and about the F strain of HSV-1 (4, 10, 15), which was also used in this study.

HSV-1 and HSV-2 virions were purified by centrifugation of cytoplasmic extracts from infected HEp-2 cells on 36-ml gradients of dextran 10 (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.), in which the density increased linearly from 1.04 to 1.09 g/cm^3, as previously reported for the purification of HSV-1 virions (18). Some modifications of the original procedure were introduced: (i) HEp-2 cells in roller bottles were infected at 3 to 5 PFU/cell and incubated at 34 C for 48 h before harvest, (ii) the infected cells were scraped directly into the tissue culture medium and collected by centrif-

ugation, omitting any washes of the cells with phosphate-buffered saline. For preparation of the cytoplasmic extracts, approximately 4×10^8 infected cells (radiolabeled as described in the legends to Fig. 1 and 2) were suspended in 1.5 ml of 1 mM phosphate buffer (pH 7.4) and, after 10 min at 0 C, were disrupted in a Dounce homogenizer. The Dounce homogenization was carefully monitored by phase microscopy to ensure that all cells were disrupted with minimal nuclear breakage. Nuclear pellets, obtained after centrifugation of the homogenate for 10 min in a clinical centrifuge, were washed once with 1.5 ml of 1 mM phosphate buffer. The cytoplasmic extract combined with the nuclear wash (2 to 3 ml total) was then layered on one dextran 10 gradient and centrifuged for 1 h at 20,000 rpm in an SW27 rotor. Virions sedimented to a position near the middle of the gradient and formed an opalescent band. Electron microscope observations of negatively stained preparations revealed that the opalescent bands contained enveloped virus predominantly with little or no evidence of contamination.

Inasmuch as membrane vesicles are the major possible source of contamination in this procedure (18), the electron microscope observations are an important criterion of purity and revealed that the HSV-2 preparations were as satisfactory as the HSV-1 preparations. We noted, however, that whereas the HSV-1 preparations routinely contained less than 10% unenveloped nucleocapsids, as many as 30% of the particles were not enveloped in some HSV-2 preparations. This finding is consistent with previous observations that, in HSV-2-infected cells, naked nucleocapsids are more frequently found in the cytoplasm than in HSV-1-infected cells (17). For the purpose of this communication, therefore, it must be noted that the relative amounts of polypeptides detected in HSV-2 preparations may not accurately reflect the relative quantities present in virions.

For analyses of the virion proteins by SDS-acrylamide gel electrophoresis, the virus obtained from dextran gradients was diluted at least four-fold with 0.01 M Tris-hydrochloride, pH 7.4, and collected by centrifugation at 25,000 rpm for 2 h in the SW27 rotor. The virus pellets were dissolved in a solution containing SDS and subjected to electrophoresis on 8.5% acrylamide gels cross-linked with *N,N'*-diallyltartardiamide as previously described (7).

Figure 1 shows the electrophoretic profiles of [^{35}S]methionine-labeled or [^{14}C]glucosamine-labeled proteins from HSV-1(F) and HSV-2(G) virions. In Fig. 2 are shown the [^{14}C]amino acid-labeled proteins present in virions from each of the five different HSV-2 isolates. The numbers assigned to individual HSV-1(F) polypeptide bands in Fig. 1 follow the designations

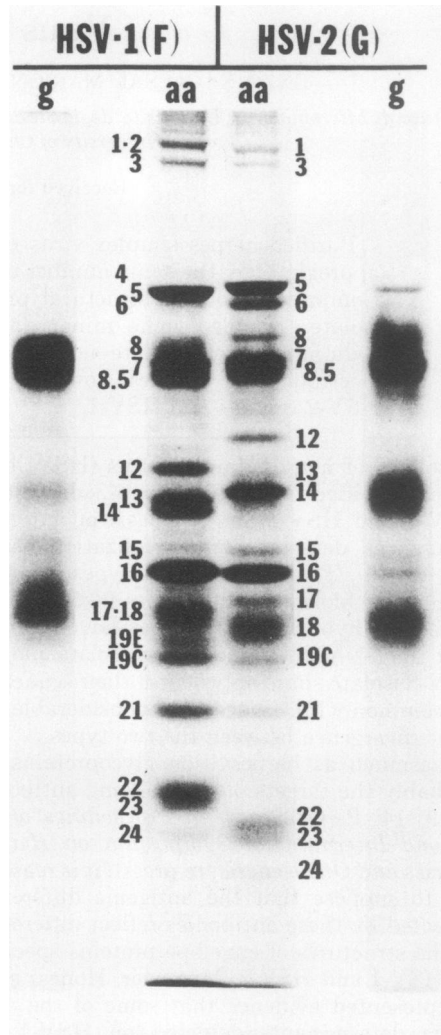

FIG. 1. *Autoradiogram of an SDS-acrylamide gel slab showing the [^{35}S]methionine-labeled (aa) or [^{14}C]glucosamine-labeled (g) polypeptides present in HSV-1(F) and HSV-2(G) virions. For preparation of the radiolabeled virus, HEp-2 cells were incubated from 5 to 48 h postinfection in medium 199 containing 1% calf serum, 1/10 the usual level of methionine, and 1 μCi of [^{35}S]methionine (100-400 Ci/mmol; New England Nuclear, Boston, Mass.) per ml or in medium 199 containing 1% calf serum, 1/2 the usual level of glucose, and 0.75 μCi of [^{14}C]glucosamine (45 to 55 mCi/mmol; New England Nuclear, Boston, Mass.) per ml.*

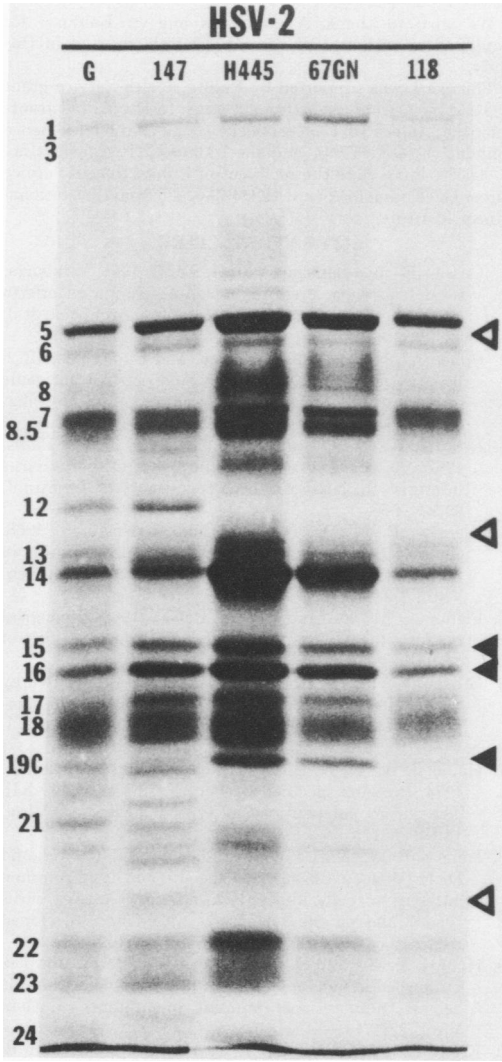

FIG. 2. *Autoradiogram of an SDS-acrylamide gel slab showing the [^{14}C]amino acid-labeled polypeptides present in virions from five independent isolates of HSV-2. The infected HEp-2 cells from which the virions were isolated were incubated in radioactive medium as described in the legend to Fig. 1 except that a mixture of [^{14}C]amino acids (leucine, isoleucine, and valine) was used at a total activity of 0.3 μCi per ml of medium 199. The triangles mark the positions to which several electrophoretically invariant HSV-1 polypeptides migrate in relation to the profiles of the HSV-2 polypeptides. The HSV-1 polypeptides designated 5, 12, and 22 (◁) do not have identical counterparts in most or all of the HSV-2 virions, whereas species similar in electrophoretic mobility to HSV-1 polypeptides 15, 16, and 19C (◀) are detected in all the HSV-2 isolates.*

ide cross-linker for acrylamide gels was introduced (7). Use of the *N,N'*-diallyltartardiamide cross-linker instead of the usual bis-acrylamide allows better resolution of certain polypeptides, particularly the glycoproteins, but changes the relative mobilities of glycoproteins in bands 7, 8, and 8.5. Numbers have been assigned to individual HSV-2(G) polypeptides in Fig. 1 and 2 for convenience in discussion.

It is apparent from the profiles shown in Fig. 1 and 2 that HSV-2 virions contain approximately the same number of polypeptide species as HSV-1 virions. We can infer that, for both serotypes, most of the virion proteins are located in the envelope because Gibson and Roizman (6) previously demonstrated that the naked nucleocapsids of HSV-1(F) and HSV-2(G) each contain only six proteins at most. It is also apparent that some of the polypeptides found in HSV-1(F) virions are not present in identical form in HSV-2 virions and vice versa. A recent study of the structural polypeptides specified by 53 different HSV-1 strains revealed that certain polypeptide bands with characteristic electrophoretic mobilities in SDS gels were detected in all the strains [including HSV-1(F)], whereas variability was observed in the migration rates of seven prominent species (L. Pereira, E. Cassai, R. W. Honess, B. Roizman, M. Terni, and A. J. Nahmias, submitted for publication). Of the electrophoretically invariant HSV-1 polypeptides, at least three (designated 5, 12, and 22 in Fig. 1) were not detected in identical form in any of the HSV-2 isolates analyzed in this study. The positions of these HSV-1 polypeptides are marked by the open triangles in Fig. 2. Although HSV-2 virions probably contain functional counterparts to these three proteins, only one can be identified on the basis of available data. Studies by Gibson and Roizman (6) and by Honess and Roizman (unpublished data) revealed that polypeptide 5 of HSV-1 and 5 of HSV-2 (Fig. 1) are the major capsid proteins of HSV-1(F) and HSV-2(G) nucleocapsids, respectively. Our studies demonstrate that the major capsid proteins of five different HSV-2 isolates have similar or identical electrophoretic mobilities (Fig. 2) but migrate slightly slower than the major capsid protein of HSV-1(F) and of all the other HSV-1 isolates studied to date (Pereira et al., submitted for publication).

Figure 1 also demonstrates that some of the HSV-2(G) glycoproteins differ from HSV-1(F) glycoproteins, at least in relative amounts of glucosamine incorporation or in electrophoretic mobility. This observation, although consistent with the body of data in support of structural

differences between the HSV-1 and HSV-2 glycoproteins (reviewed by P. G. Spear, *in* H. A. Blough and J. M. Tiffany [ed.], *Cell Membranes and Viral Envelopes*, in press), cannot readily be interpreted because there is no theoretical basis for predicting the electrophoretic behavior of glycoproteins in SDS gels. The possibility exists that none of the HSV-2 glycoproteins is identical in structure to any of the HSV-1 glycoproteins; recent studies (P. G. Spear, *in Proceedings of the Second International Symposium on Herpesviruses and Oncogenesis*, in press; M. Sarmiento and P. G. Spear, manuscript in preparation) demonstrate, however, that most of the HSV-2(G) glycoproteins are antigenically related to HSV-1(F) glycoproteins.

The electrophoretic analyses shown in Fig. 2 reveal very few differences in the structural proteins specified by the five HSV-2 isolates. The most obvious one is that G and 147 contain band 12, whereas the other three isolates lack an identical form of this protein; there are also variations in some of the smaller-molecular-weight proteins. On the other hand, all of the HSV-2 isolates have structural polypeptides migrating in the positions of bands 1, 5, 6, 7, 14, 15, 16, 17, 18, 19C, 22, and 23. Polypeptides having electrophoretic mobilities similar to 15, 16, and 19C (marked by the filled triangles in Fig. 2) have also been detected in each of 53 HSV-1 strains (Pereira et al., submitted for publication). Band 19C was previously identified as a component of both HSV-1 (F) and HSV-2 (G) nucleocapsids (6), whereas bands 15 and 16 are probably nonglycosylated envelope proteins.

In summary, comparisons of virions from five HSV-2 isolates revealed striking similarities in the electrophoretic profiles of their structural polypeptides on SDS-acrylamide gels. The HSV-2 isolates could be differentiated from HSV-1, however, on the basis of the electrophoretic mobilities of certain polypeptides that appear to be characteristic of each serotype. Specifically, the major capsid protein of HSV-1 migrates slightly faster than that of HSV-2. Moreover, two prominent HSV-1 polypeptide bands, designated 12 and 22, have electrophoretically similar counterparts in all HSV-1 isolates examined to date but not in any of the HSV-2 isolates analyzed in this study. Differences in the electrophoretic mobilities of SDS-solubilized nonglycosylated proteins reflect differences in size and therefore in structure. Consequently, the work reported here provides a starting point for identifying HSV-1 or HSV-2 proteins that might differ sufficiently to be useful as type-specific antigens.

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