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Comparative Structural Analysis of Glycoprotein gD of Herpes Simplex Virus Types 1 and 2

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We studied the synthesis and processing of the type-common glycoprotein gD in herpes simplex virus type 2 (HSV-2) and compared it structurally to glycoprotein gD of herpes simplex virus type 1 (HSV-1). We demonstrated that in HSV-2, gD undergoes posttranslational processing from a lower-molecular-weight precursor (pgD51) to a higher-molecular-weight product (gD56). Tryptic peptide analysis by cation-exchange chromatography indicated that this processing step altered neither the methionine nor the arginine tryptic peptide profile of gD of HSV-2. Comparative tryptic peptide analysis of gD of HSV-1 and HSV-2 showed that the methionine and arginine tryptic peptide profiles of these two proteins were very similar, but not identical. Some of the resolved peptides coeluted from the cation-exchange column, suggesting that some amino acid sequences of the two proteins might be very similar. However, each protein also appeared to possess several type-specific tryptic peptides. The structural similarity of these two glycoproteins correlates well with their antigenic cross-reactivity since monoprecipitin antibody to gD of HSV-1 also immunoprecipitates gD of HSV-2 and neutralizes the infectivity of both viruses to approximately the same extent.

Herpes simplex virus (HSV) appears to contain five glycoproteins which have been designated gA, gB, gC, gD, and gE (1). These molecules differ in electrophoretic mobility in sodium dodecyl sulfate (SDS) polyacrylamide gels (3, 8, 14, 16, 19, 22, 26), in biological properties (1, 13, 23, 28), and in genetic map positions (14, 22).

Previously, we described an HSV-specific antigen, CP-1 (5, 7), purified from HSV type 1 (HSV-1)-infected cells and having the properties of a glycoprotein. This glycoprotein stimulated production of high titers of type-common neutralizing activity. Recently (5, 8), we showed that CP-1 is associated in infected cells with two components, a 52,000-molecular-weight (52K) precursor (pgD 52) and a 59K product (gD59). Furthermore, we recently established by tryptic peptide analysis that pgD52 and gD59 of HSV-1 shared methionine and arginine tryptic peptides (8). In addition, gD found in cytoplasmic extracts of HSV-1-infected cells has the same methionine peptide profile as gD isolated from the virion. Processing of pgD52 to gD59 involved addition of carbohydrate and especially sialic acid residues to the protein, and this latter addition altered the charge and the molecular weight of the molecule (8; manuscript in preparation).

Although HSV-1 differs from HSV-2 in a number of biological, biochemical, and biophysical properties (15, 20), the two types also exhibit similarities. DNA-DNA hybridization studies indicate that the DNAs of HSV-1 and HSV-2 exhibit approximately 50% homology with 85% matching of base pairs of the homologous regions (12). Whereas the glycoproteins of HSV-2 appear in the same general molecular-weight range in SDS polyacrylamide gel electrophoresis (PAGE) as those of HSV-1, what appears to be glycoprotein gD of HSV-2 is slightly smaller in molecular weight than glycoprotein gD of HSV-1 (3, 14, 19, 22, 25). Studies with intertypic recombinants suggest that gD from HSV-1 and gD from HSV-2 map in approximately the same position on the unique short segment of the viral genome (14, 22). In addition, we found that monoprecipitin antibody to gD of HSV-1 neutralizes the infectivity of HSV-1 and HSV-2 to approximately the same extent (5).

Almost all of the work establishing precursor-product relationships among HSV glycoproteins has been conducted on HSV-1. In the present study, we extended this type of analysis to HSV-2. The purpose of the present studies was two-fold: (i) to establish whether a precursor-product relationship similar to that found for gD of HSV-1 exists for gD of HSV-2, and (ii) to determine whether gD of HSV-2 is structurally similar to gD of HSV-1.

We present evidence that the precursor pgD51

and product gD56 forms of gD of HSV-2 share methionine and arginine tryptic peptides. Processing of gD of HSV-2 does not appear to involve changes in the polypeptide portion of the molecule. Detailed tryptic peptide analyses of gD of HSV-1 and gD of HSV-2 indicate that the molecules are very similar, though not identical, and appear to have several tryptic peptides in common. Each protein also appears to possess several type-specific tryptic peptides.

MATERIALS AND METHODS

Cell cultures. Conditions for the growth and maintenance of BHK cells have been previously described (7).

Virus preparation and titration. The procedures used for the preparation of virus stocks of HSV-1 (strain HF) and HSV-2 (Savage strain) and the plaque assay were described previously (4, 7). For infection, unless otherwise noted, an input multiplicity of 20 PFU of HSV-1 or 10 PFU of HSV-2 per cell was employed.

Pulse-chase experiments. Previous studies of this kind using HSV-1 were done with KB cells (5, 8). However, the Savage strain of HSV-2 does not yield high titers of virus in KB cells. In other studies (8, 17), we established that the methionine and arginine tryptic fingerprint profiles of gD from HSV-1 are the same in BHK or KB cells. Nevertheless, to avoid any problems in comparing gD of HSV-1 with gD of HSV-2 that might have arisen as a result of differences in cell type, we performed all pulse-chase experiments in this study on BHK cells by a modification of methods previously described for KB cells (5, 8). To increase the incorporation of isotopically labeled methionine or arginine, the cells (35-mm plates) were overlaid after infection with Eagle minimal medium containing 1/10 the normal concentration of methionine or arginine. Pulse-labeling with methionine or arginine was carried out by incubating infected cells in 0.5 ml of Hanks salts containing one of the following radioisotopes: [^{35}S]methionine (specific activity, 600 Ci/mmol), 200 μCi; [*methyl*-^{3}H]methionine (specific activity, 100 Ci/mmol), 250 μCi; [*U*-^{14}C]arginine (specific activity, 336 mCi/mmol), 125 μCi; [2,3-^{3}H]arginine (specific activity, 15 Ci/mmol), 125 μCi. For incorporation of labeled mannose, infected cells were pulse-labeled in 0.5 ml of Eagle minimal medium minus serum and glucose with 500 μCi of [2-^{3}H]mannose (specific activity, 18 Ci/mmol). After a 15-min pulse, the label was removed, and the monolayers were either washed with iced saline and immediately frozen at −70°C (pulse) or incubated for an additional 4 h in prewarmed complete medium (chase). After the pulse and chase, the cells were lysed, and cytoplasmic extracts were prepared as described previously (5, 8). Extracts were stored at −70°C. All radioisotopes were purchased from New England Nuclear Corp.

Virus purification. HSV-1 was purified by the method of Spear and Roizman (27). Cells were harvested for virus purification at 18 h postinfection (p.i.). To solubilize the HSV envelope, purified virions were suspended in 0.02 M Tris buffer (pH 7.5) containing 0.15 M NaCl and Nonidet P-40 (NP-40) and incubated at 25°C for 1 h. L-1-Tosylamide-2-phenethyl chloromethyl ketone and *N*-2-*p*-tosyl-L-lysine chloromethyl ketone hydrochloride were added, each at a concentration of 0.1 mM, to inhibit proteolytic activity. Nucleocapsids were removed by centrifugation at 100,000 × *g* for 2 h. The resulting pellet was reextracted with NP-40 and recentrifuged. The supernatants from both extractions were pooled and frozen at −70°C.

Immunological studies. The preparation of antisera against HSV-1 used in these studies was previously described (5, 7). Anti-ENV-1 serum was prepared against an NP-40 extract of the virion envelope of HSV-1. Anti-CP-1 serum was prepared against a purified preparation of gD of HSV-1 (5). Anti-ENV-2 serum was prepared against an NP-40 extract of virion envelopes of HSV-2 by the regimen previously described (7). Anti-ENV-2 serum had a serum neutralization titer (50% reduction of PFU [18]) of 1,024 against HSV-2 and 256 against HSV-1.

For antibody precipitation, *Staphylococcus aureus* Cowan strain I (IgSorb; New England Enzyme Center) was employed to collect antigen-antibody complexes (11, 17, 24). Each antiserum was titrated to ensure that the maximal amount of antigen was precipitated. The precipitates were washed, and the antigen-antibody complexes were dissociated as described previously (17).

SDS-PAGE. SDS-PAGE was carried out in slabs of 10% acrylamide cross-linked with 0.4% *N,N'*-diallyltartardiamide by essentially the same method described by Spear (25). After electrophoresis, the gels were stained with Coomassie brilliant blue (5), dried on filter paper, and exposed to Kodak X-Omat R (XR-5) film. To locate tritium-labeled bands for tryptic peptide analysis, the dried gels were exposed to LKB Ultrafilm (LKB Instruments, Inc.). For fluorography, the procedure of Bonner and Laskey (2) was followed. Protein standards ranging from 15,000 to 130,000 daltons were run on each gel (5).

Preparation of samples for tryptic peptide analysis. *N,N'*-diallyltartardiamide gel slices were dissolved in 2% periodic acid by the method of Gibson (9) and prepared for trypsinization by methods described previously (8). Trypsinization and ion-exchange chromatography were carried out as previously described (8, 29), except that in certain cases, a modification was made in the buffer system, which helped to resolve peptides that eluted at the more basic end of the pH gradient. For purposes of clarity, the buffer system described previously (8) will be called buffer system 1, and the modified buffer system will be called buffer system 2. In buffer system 1, buffer A consisted of 280 ml of acetic acid, 4 ml of pyridine, and 716 ml of water (8). In buffer system 2, buffer A consisted of 310 ml of acetic acid, 4 ml of pyridine, and 716 ml of water. In both buffer systems, buffer B consisted of 143 ml of acetic acid, 161 ml of pyridine, and 696 ml of water. Three mixing chambers were used to generate the gradient (8); chambers 1 and 2 each contained 180 ml of buffer A, and chamber 3 contained 60 ml of buffer A plus 120 ml of buffer B. The arrows on the Fig. 2, 3, 5, and 6 indicate where elution with buffer B alone (basic wash) started.

RESULTS

SDS-PAGE of HSV-2 glycoproteins. In previous experiments with HSV-1 infection of KB cells, we established that synthesis and processing of glycoproteins occurred optimally between 5 and 8 h p.i. (unpublished data). In the current experiments employing BHK cells, we found that this time was also optimal for HSV-1 or HSV-2 glycoprotein synthesis and processing. More specifically, synthesis and processing of pgD51 of HSV-2 could be detected as early as 2 h p.i., and maximal synthesis of this precursor occurred by 4 to 6 h p.i. Therefore, we chose 5 h p.i. for pulse-labeling of both HSV-1- and HSV-2-infected BHK cells.

Figure 1 shows the electrophoretic pattern of [^{3}H]arginine-labeled proteins (tracks 1, 2, 5, and 6) or [2-^{3}H]mannose-labeled proteins (tracks 3 and 4) immunoprecipitated from cytoplasmic extracts of HSV-2-infected cells after a 15-min pulse at 5 h p.i. or after a 15-min pulse at 5 h p.i. followed by a 4-h chase. Two antisera were used in this experiment: a homologous antiserum, anti-ENV-2, prepared against an NP-40 extract of virion envelope of HSV-2 (tracks 1 through 4) and a heterologous serum, anti-CP-1, prepared against gD of HSV-1 (5–8) (tracks 5 and 6). Tracks 1, 3, and 5 show that a 51K protein was immunoprecipitated from the pulse-labeled cytoplasmic extract by either serum. Tracks 2, 4, and 6 show that these same sera immunoprecipitated a larger-molecular-weight form (56K) from the cytoplasmic extract of the chase. Essentially the same results were obtained when [^{3}H]methionine or [^{35}S]methionine was used as the label. Since [^{3}H]mannose was incorporated into the 51K and 56K forms (tracks 3 and 4), it appears that both of these molecules were glycosylated. Furthermore, monoprecipitin antiserum to gD of HSV-1 reacted with both the 51K and 56K forms of gD of HSV-2 (tracks 5 and 6). Taken together, the results of this experiment show that the 51K and 56K proteins of HSV-2 are antigenically related and probably represent precursor (pgD51) and product (gD56) forms of the same glycoprotein. Furthermore pgD51 and gD56 are both antigenically related to gD of HSV-1 (tracks 5 and 6). It should also be noted that anti-ENV-2 serum immunoprecipitated several other bands from the pulse-labeled extract with molecular weights of approximately 63K, 65K, 105K, and 127K. These bands appeared to be replaced in the chase by a set of bands of 73 to 97K and 110K. Since mannose was also incorporated into these polypeptides, it appears that all of these bands represent glycoproteins. These polypeptides were not investigated further.

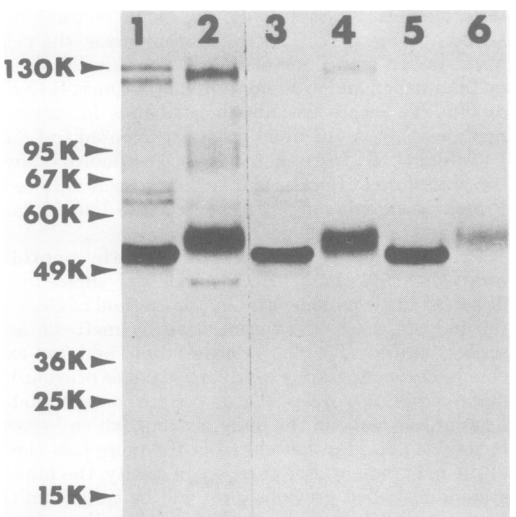

FIG. 1. *SDS-PAGE analysis of HSV-2 glycoproteins. Fluorogram of a 10% N,N'-diallyltartardiamide cross-linked polyacrylamide gel of immune precipitates obtained from lysates of HSV-2-infected BHK cells. Track 1, [^{3}H]arginine pulse, anti-ENV-2 serum; track 2, [^{3}H]arginine chase, anti-ENV-2 serum; track 3, [2-^{3}H]mannose pulse, anti-ENV-2 serum; track 4, [2-^{3}H]mannose chase, anti-ENV-2 serum; track 5, [^{3}H]arginine pulse, anti-CP-1 serum; track 6, [^{3}H]arginine chase, anti-CP-1 serum. Tracks 1 and 2 were exposed to Kodak X-Omat R (XR-5) film for 1 day. Tracks 3 through 6 were exposed to film for 3 days.*

Tryptic peptide analysis of pgD and gD of HSV-2. To establish that pgD51 and gD56 of HSV-2 were structurally related, we compared the two polypeptides by tryptic peptide analysis.

Cytoplasmic extracts labeled with [^{35}S]methionine or [^{3}H]methionine were immunoprecipitated and analyzed by SDS-PAGE. The bands corresponding to pgD51 and gD56 were eluted, trypsinized, and cochromatographed on a Chromobeads P (Technicon Corp.) column. Figure 2 shows that the elution profiles for pgD51 and gD56 were identical. For both polypeptides, seven distinct methionine-labeled peptide peaks were eluted from the column; one peak was the flow-through (fractions 1 through 6), three minor peaks eluted in the acidic region (fractions 30 through 50), two major peaks eluted in the middle portion of the column, and one peak eluted with the basic wash. These results indicate that pgD51 and gD56 of HSV-2 are structurally related.

To obtain a more complete structural comparison of pgD51 and gD56, we examined the arginine profiles of the two proteins. Figure 3 shows that pgD51 and gD56 shared essentially all resolved arginine peptides. Moreover, since a

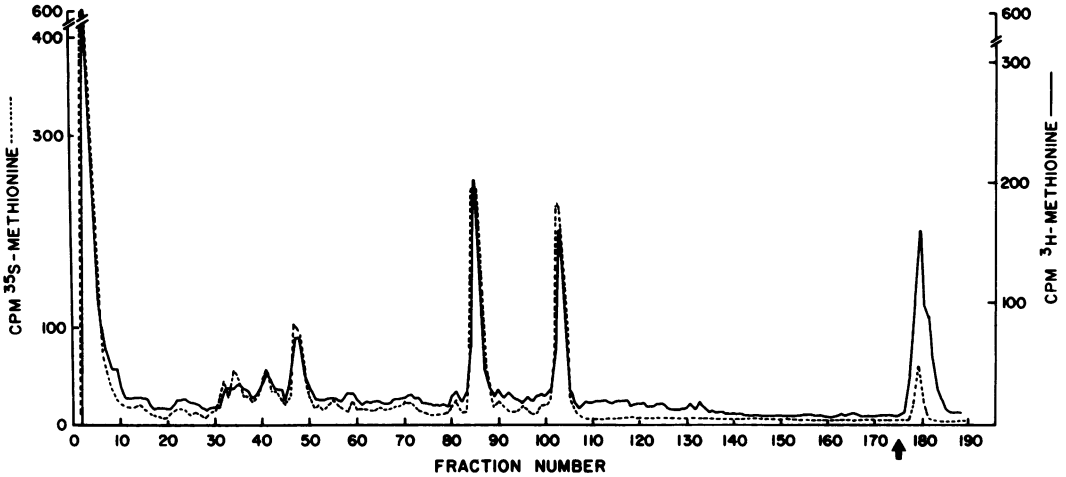

FIG. 2. *Tryptic fingerprint analysis of glycoprotein gD of HSV-2. pgD51 (——) isolated by immunoprecipitation and SDS-PAGE from [^{3}H]methionine pulse-labeled extracts (15 min at 5 h p.i.) was cochromatographed on a column of Chromobeads P with [^{35}S]methionine-labeled gD56 (-----) isolated in a similar fashion from the chase (15-min pulse at 5 h p.i., followed by a 4-h chase). Fraction size 2.2 ml, buffer system 1 (see the text).*

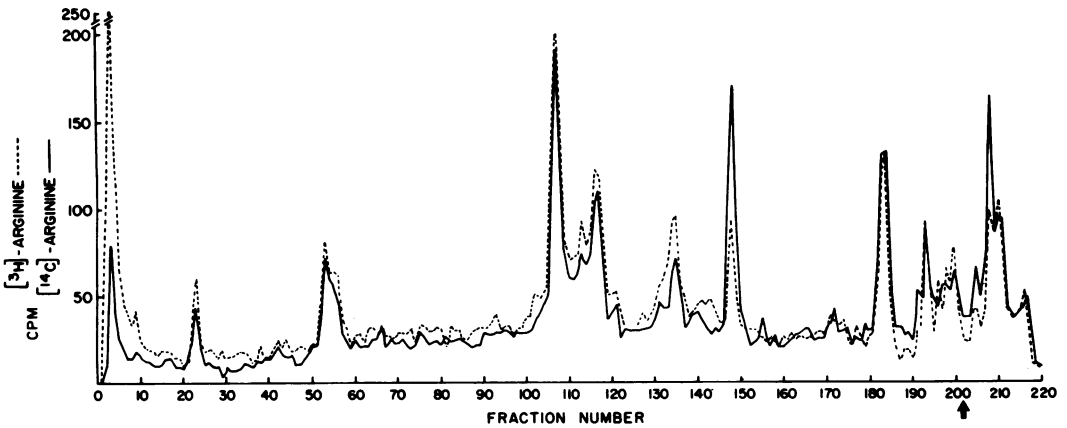

FIG. 3. *Tryptic fingerprint analysis of glycoprotein gD of HSV-2. pgD51 (——) isolated by immunoprecipitation and SDS-PAGE from [^{14}C]arginine pulse-labeled extracts (15 min at 5 h p.i.) was cochromatographed on a column of Chromobeads P with [^{3}H]arginine-labeled gD56 (-----) isolated in a similar fashion from the chase (15-min pulse at 5 h p.i., followed by a 4-h chase). Fraction size 2 ml, buffer system 2 (see the text).*

large number of arginine peptides were resolved, it is likely that a fairly substantial portion of the amino acid sequence in the two polypeptides was identical. Thus, we concluded that the processing of pgD51 to gD56 in HSV-2 does not entail any major alteration in the peptide portion of the molecule.

Comparison of gD of HSV-1 and HSV-2. (i) Immunological similarities. We showed in Fig. 1 that monoprecipitin antibody to gD of HSV-1 (5, 8) also acted as a monoprecipitin antibody to pgD51 and gD56 of HSV-2. Figure 4 compares pgD51 of HSV-2 (track 1) with pgD52 of HSV-1 (track 2) by SDS-PAGE. These results showed directly that the type 1 precursor of gD has a slightly higher molecular weight than the type 2 precursor. This difference has been noted previously (3, 14, 22, 26) and has been used as the basis for distinguishing the two proteins in intertypic recombinants (14, 22). Previously (5), we showed that anti-CP-1 serum neutralized the infectivity of HSV-2 to approximately the same extent as HSV-1, suggesting that some of the sites on pgD involved in the initial stages of infection were similar in structure.

(ii) Structural similarities. The precursor forms of glycoprotein gD of HSV-1 and HSV-2 were isolated by SDS-PAGE, oxidized, trypsinized, and cochromatographed on a column of

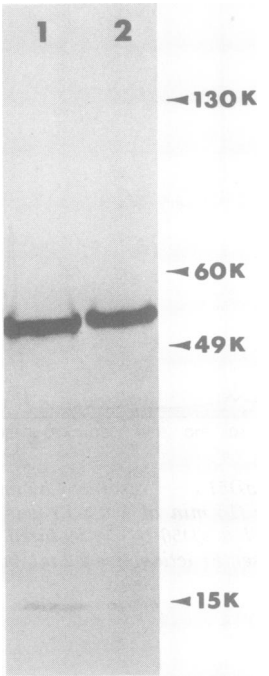

FIG. 4. *SDS-PAGE analysis of glycoprotein gD of HSV-1 and HSV-2. Fluorogram of a 10% N,N'-diallyltartardiamide cross-linked polyacrylamide gel of immune precipitates obtained from pulse-labeled extracts of HSV-1 and HSV-2. Track 1, [^{3}H]arginine pulse, HSV-2-infected cells, anti-CP-1 serum; track 2, [^{3}H]arginine pulse, HSV-1-infected cells, anti-CP-1 serum.*

Chromobeads P. Figure 5 shows the elution profile of [^{3}H]methionine-labeled pgD52 of HSV-1 cochromatographed with [^{35}S]methionine-labeled pgD51 of HSV-2. The results show that the gD glycoproteins of HSV-1 and HSV-2 shared one major methionine-labeled peptide peak (fraction 107, pH 3.33). The second major methionine-labeled peak seen for pgD51 of HSV-2 (fraction 90) was absent from pgD52 of HSV-1. The pattern for pgD52 of HSV-1 agrees with previous results (8, 17). Two type-specific peaks, one for pgD of HSV-1 (fraction 35) and one for pgD of HSV-2 (fraction 50), were resolved in this experiment. We concluded that the gD glycoproteins of HSV-1 and HSV-2 have one methionine-containing peptide peak in common (pH 3.33) and three that are different.

In the next experiment (Fig. 6), pgD51 of HSV-2 labeled with [^{3}H]arginine was cochromatographed with pgD52 of HSV-1 labeled with [^{14}C]arginine. It can be seen that the elution profiles of the tryptic peptides of the two proteins were very similar throughout the gradient. In some cases, the peaks overlapped completely (e.g., peaks at fractions 99, 115, 131, 167, 175, 181, and 185). In other instances, arginine peaks for the two virus types eluted very closely but did not overlap completely. An interesting observation about these similar but nonoverlapping peaks is that in every case, the peak for HSV-2 eluted at a slightly more acidic pH (to the left) than did the corresponding peak for HSV-1. In a few cases, notably at fractions 30, 40, 126, and possibly 140, there were peaks resolved for pgD52 of HSV-1 that were missing from the profile of pgD51 of HSV-2. These four peaks thus appear to represent HSV-1-specific arginine peptides.

Our conclusion from these experiments is that the methionine and arginine peptide profiles of gD of HSV-1 and HSV-2 are very similar. Several tryptic peptides coeluted, suggesting that they may be structurally similar. However, the two glycoproteins are not identical since each contained several type-specific peptides.

DISCUSSION

The studies presented in this paper establish two properties of glycoprotein gD. First, the protein undergoes posttranslational processing in HSV-2-infected cells from a lower-molecular-weight precursor (pgD51) to a higher-molecular-weight product (gD56) in a manner which does not appear to alter its methionine or arginine tryptic peptide profile. The processing of gD of HSV-2 thus closely resembles that of gD of HSV-1 (8). The second property of gD established in this paper is that the methionine and arginine tryptic peptide profiles of gD of HSV-1 and HSV-2 appear to be very similar but not identical. Some of the resolved tryptic peptides of the two proteins coeluted during chromatography, suggesting that their amino acid sequences are quite similar or identical. Thus, the intertypic structural similarities correlate well with the immunological cross-reactivity of anti-CP-1 serum (5). Other investigators have noted a similar cross-reactivity (10, 25). It might be interesting to determine whether the antigenic sites responsible for inducing neutralizing antibody are structurally identical in the two proteins and whether, in fact, they correspond to one or more of the resolved tryptic peptides. Assuming that gD participates in the absorption phase of virus entry, as suggested by Spear et al. (28), studies of the structure of the specific peptides involved in this process in HSV-1 and HSV-2 may shed some light on the nature of the cell receptor.

Although our studies suggest that there may be considerable intertypic structural homology of gD, they do not allow us to ascertain the

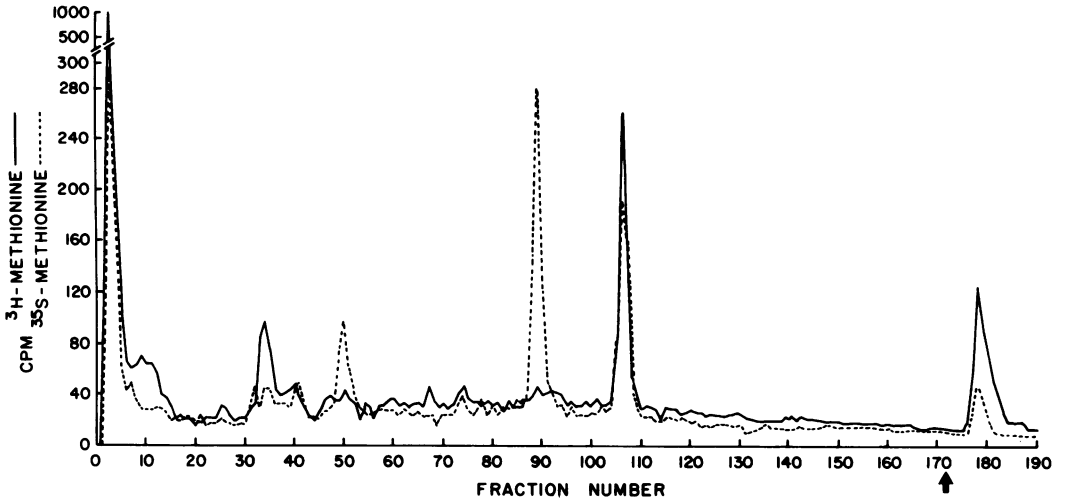

FIG. 5. *Comparative tryptic fingerprint analysis of glycoprotein gD of HSV-1 and HSV-2. pgD52 isolated by immunoprecipitation and SDS-PAGE from [³H]methionine pulse-labeled extracts of HSV-1-infected cells (——) was cochromatographed on Chromobeads P with pgD51 isolated in a similar fashion from [³⁵S]-methionine pulse-labeled extracts of HSV-2-infected cells (-----). Fraction size 2.2 ml, buffer system 1 (see the text).*

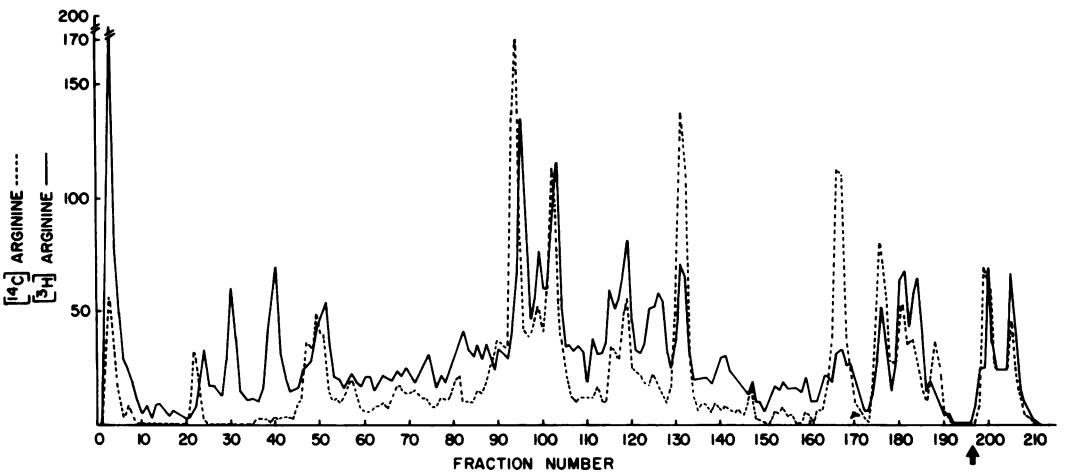

FIG. 6. *Comparative tryptic fingerprint analysis of glycoprotein gD of HSV-1 and HSV-2. pgD52 isolated from [³H]arginine pulse-labeled extracts of HSV-1-infected cells (——) was cochromatographed on Chromobeads P with pgD51 isolated from [¹⁴C]arginine pulse-labeled extracts of HSV-2-infected cells (-----). Fraction size 2.0 ml, buffer system 2 (see the text).*

extent of this homology. Other properties of these glycoproteins offer some additional clues about the extent of structural relatedness. For example, studies with intertypic recombinants have shown that HSV-1 strains are infectious when they contain a type 2 gD and vice versa (14, 22). This means that the two glycoproteins are functionally similar and that any structural differences that do exist, even in the site for absorption, are not important enough to prevent infection by the recombinant strain. In this regard, some estimates of the number of type-common and type-specific antigenic sites present in gD were made by Halliburton et al. (10). In those studies, monoprecipitin antibody to band II (corresponding to gD) was made HSV-1 specific by absorption with the heterologous virus. This serum was capable of neutralizing type 1 parents and recombinants containing gD from HSV-1 but not type 2 parents or recombinants. The data of Halliburton et al. imply that at least one of the antigenic sites involved in inducing neutralizing antibody is not identical in gD of HSV-1 and HSV-2.

In the course of our pulse-chase experiments, we noted that the apparent difference in the molecular weight of the product glycoproteins (gD56 and gD59) was greater than the difference in the molecular weight of the precursors (pgD51 and pgD52). Since there was no apparent alteration of the polypeptide chain during processing of either glycoprotein, it is possible that the greater apparent molecular weight change in processing of gD of HSV-1 might be due to addition of more or different carbohydrates during processing from the precursor, pgD, to the product, gD. We had previously shown (8) that processing of gD59 in HSV-1 involved addition of an 1,800-molecular-weight core oligosaccharide (high-mannose type) to the core polypeptide, possibly a 50K molecule (17), to form pgD52. This was followed by another step of processing in which the core oligosaccharide was modified by addition of other carbohydrates, including sialic acid, to form a complex-type oligosaccharide. A similar processing pattern probably occurs for gD of HSV-2. One explanation for the smaller molecular weight increase in the processing of gD of HSV-2 is that some of the oligosaccharide side chains of this molecule are not converted to the complex type. For closely related murine leukemia viruses, slight differences in protein structure apparently determine whether or not a particular oligosaccharide side chain will undergo processing from the high-mannose type to the complex type (21). Thus, slight differences in amino acid sequence might account for the difference in the apparent molecular weight of gD56 and gD59.

This explanation would not account for the smaller molecular weight difference of the two precursors pgD51 and pgD52. It is possible that gD of HSV-1 is 8 to 10 amino acids larger than gD of HSV-2. Alternatively, this difference could be due to the number of attached oligosaccharide chains. For instance, gD of HSV-2 might contain one less asparagine-linked oligosaccharide than gD of HSV-1. This might be due to substitution of another amino acid for asparagine. In fact, if gD of HSV-2 contained fewer glycosylation sites, there would be fewer sites available for sialylation. Processing of pgD51 to gD56 would therefore involve a smaller molecular weight change than processing of pgD52 to gD59 even if all of the oligosaccharides of the two proteins were processed similarly.

These differences in oligosaccharides are all consistent with the idea that gD glycoproteins of HSV-1 and HSV-2 are very similar in amino acid sequence. Preliminary evidence suggests that the arginine peptides present in gD of HSV-1, but not in gD of HSV-2, can be partially accounted for by type-specific lysine peptides in gD of HSV-2. Thus, some of the intertypic differences in the arginine peptide profiles shown in Fig. 6 could arise as the result of a single base change, e.g., AAA (lysine) to AGA (arginine) or AAG (lysine) to AGG (arginine). Of course, all of this is speculative, and the actual extent of structural homology can only be determined by more detailed analysis of both the carbohydrate and peptide portions of the proteins.

Our previous studies of the capsid proteins of HSV-1 and HSV-2 led us to conclude that none of the analogous structural proteins of HSV-1 and HSV-2 were identical, although two of them (the major capsid protein NC-1 and the smallest capsid protein NC-7) appeared to be very similar by tryptic peptide analysis (6). gD represents the sixth HSV structural protein examined for intertypic similarities by tryptic peptide analysis (6) and the third one which appears to be highly conserved in the two virus types.

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