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Purification of Glycoprotein gD of Herpes Simplex Virus Types 1 and 2 by Use of Monoclonal Antibody

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Glycoproteins gD-1 and gD-2 of herpes simplex virus types 1 and 2, respectively, were purified on an immunoadsorbent consisting of the type-common monoclonal antibody HD-1 linked to Sepharose. Each glycoprotein was of sufficient purity, quantity, and biological activity to be used for immunological and biochemical studies. Each glycoprotein induced high titers of type-common monospecific neutralizing antibody in mice. Amino acid analysis indicated that gD-1 and gD-2 had similar though not identical amino acid compositions.

In previous studies, we examined herpes simplex virus type 1 (HSV-1) glycoprotein gD (gD-1), including its isolation, purification, and synthesis in infected cells (4, 6, 19). A series of chromatographic steps was used to purify native gD-1 in quantities sufficient to develop a monospecific (or polyclonal) anti–CP-1 serum (4). The fact that this serum has high titers of type-common neutralizing activity indicates that gD plays an important role in the initial stages of viral infection, possibly at the level of virus attachment (21). Using anti–CP-1 serum as an immunological probe, we previously demonstrated that gD-1 and gD of HSV-2 (gD-2) are each processed from a lower-molecular-weight precursor to a higher-molecular-weight product in infected cells (4, 8, 9) by the addition of oligosaccharides (5, 8). Tryptic peptide analysis previously indicated that the protein structure of gD is highly conserved but not identical in HSV-1 and HSV-2 (9). The structural similarities of the proteins imply that they are functionally similar as well.

In the present study, we utilized the monoclonal antibody HD-1 (17), which recognizes both gD-1 and gD-2, to prepare an immunoadsorbent for the large-scale purification of both glycoproteins. Both purified glycoproteins retained antigenic activity and stimulated production of type-common neutralizing antibody. Sufficient quantities of both glycoproteins were obtained to determine their amino acid compositions.

Purification of gD by use of anti–HD-1 immunoadsorbent chromatography. A cytoplasmic extract prepared from either HSV-1–infected KB cells (4–6, 8, 9) or HSV-2–infected BHK cells and labeled with [^{35}S]methionine from 5 to 12 h postinfection (3, 9) was loaded onto an anti–HD-1 Sepharose immunoadsorbent column. The column was washed extensively (flow-through fraction) until the radioactivity reached background levels; the column was then eluted with 3 M KSCN. Figure 1 shows that the precursor and product forms of gD-2 (lane 3) and gD-1 (lane 4) were selectively bound to the HD-1 immunoadsorbent and were eluted by KSCN. The molecular weights of the glycoproteins on sodium dodecyl sulfate-polyacrylamide gels corresponded to published values (2, 4, 5, 8, 9). In the case of HSV-1, a small proportion of the label (5 to 8%) in the KSCN fraction was also found in a higher-molecular-weight compound (ca. 128,000 molecular weight [128K]; Fig. 1, lane 4). The Coomassie blue staining patterns (data not shown) of purified gD-1 (which included a 128K molecule) and gD-2 corresponded well with the radioactive patterns shown in Fig. 1 (lanes 3 and 4). No other Coomassie blue-stained polypeptides were observed in the KSCN fractions. To examine the possibility that the preparations were contaminated with low levels of host proteins, we labeled uninfected KB cells for 7 h with [^{35}S]methionine. The cytoplasmic extract (1.4×10^7 cpm) was chromatographed on HD-1 Sepharose in the same manner as that described for infected cells. No radioactively labeled or Coomassie blue-stained polypeptides were observed in the KSCN fraction. This suggests that the immunoadsorbent column did not bind host proteins to any significant extent.

Tryptic peptide analysis of purified gD-1 and gD-2. The bands corresponding to the 128K molecule, gD-1, and gD-2 were eluted from the gel (Fig. 1, lanes 3 and 4), trypsinized, and chromatographed on a Chromabeads P cation-exchange column (5, 8, 9, 22). Samples of

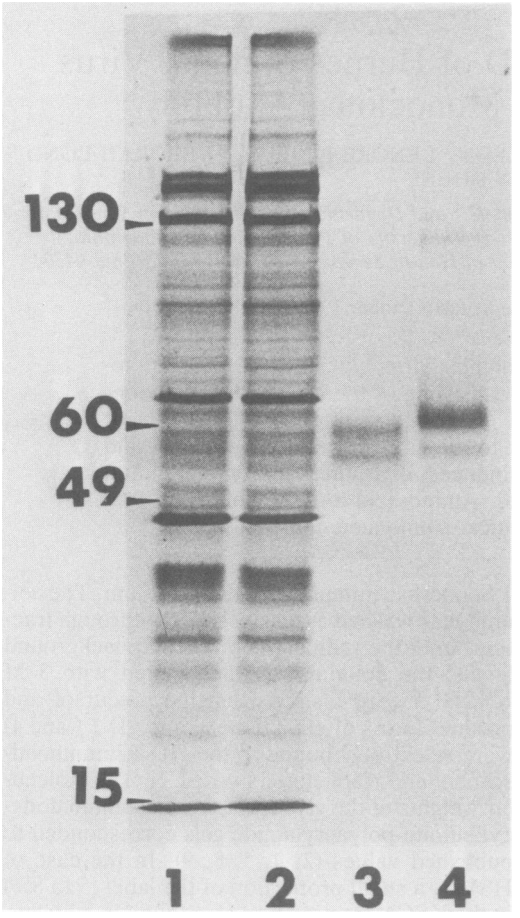

FIG. 1. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of gD-2 and gD-1 purified by immunoadsorbent chromatography: autoradiogram of a 10% diallyltartardiamide cross-linked polyacrylamide gel (9, 20). Ten roller bottles (490 cm^2) of confluent KB (3) or BHK (6) cells were infected with HSV-1 (strain HF) or HSV-2 (strain SAVAGE). At 2 h postinfection, the cells were overlaid with 50 ml of Eagle minimal essential medium containing 5% natal calf serum (Dutchland Co.). At 5 h postinfection, the medium was decanted from one of the roller bottles, and the cells were washed with warmed (37°C) Hanks salts and overlaid with 5.0 ml of Hanks salts containing 1 mCi of [^{35}S]methionine (specific activity, >600 Ci/mmol). After 30 min, the cells were overlaid with 25 ml of prewarmed complete minimal essential medium, and all of the bottles were incubated for an additional 7 h. At 12 h postinfection, labeled and unlabeled cells were washed four times with iced saline containing 0.1 mM phenylmethyl-sulfonyl fluoride (PMSF), and cytoplasmic extracts were prepared. To each roller bottle of cells, 5 ml of cold lysing buffer (0.01 M Tris buffer [pH 7.5] containing 0.15 M NaCl, 0.5% Nonidet P-40, and 0.5% sodium deoxycholate) was added, and the cells were incubated for approximately 5 min at 4°C. Tolylsulfonyl phenylalanyl chloromethyl ketone and *N*-αtosyl-L-lysine chloromethyl ketone were added, each at a concentration of 0.1 mM, to inhibit proteolytic activity. The lysed cells were scraped from the bottles and centrifuged at 1,200 rpm for 10 min to remove nuclei. The cytoplasm was centrifuged at 100,000 × *g* for 1 h. To prepare the immunoadsorbent, HD-1 IgG was purified (16) from HD-1 ascites fluid (17). Two grams of cyanogen bromide-activated Sepharose 4B (Pharmacia Fine Chemicals, Inc.) was washed according to the manufacturer's instructions and combined with 20 mg of HD-1 IgG. The immunoadsorbent was treated sequentially with ethanolamine, sodium acetate, and sodium borate according to the manufacturer's procedure. The mixture was equilibrated at 4°C with washing buffer (0.01 M Tris [pH 7.5], 0.1% Nonidet P-40, 0.5 M NaCl, 0.1 mM PMSF). For purification of gD, 55 ml of unlabeled cytoplasmic extract plus 5 ml of [^{35}S]methionine-labeled extract was added to the immunoadsorbent and recycled through the column five times. We collected 60 ml. This fraction was termed the flow-through. The column was washed overnight with washing buffer (ca. 21), and gD was eluted with 200 ml of 3 M KSCN, pH 7.8. The KSCN fraction was concentrated approximately 100-fold with an Amicon PM-30 membrane for gD-1 and a PM-10 membrane for gD-2. The concentrated sample was dialyzed extensively against a modified lysing buffer (0.01 M Tris [pH 7.5], 0.1% Nonidet P-40, 0.15 M NaCl, 0.1 mM PMSF). Lane 1, Total cytoplasmic extract of HSV-2–infected cells labeled with [^{35}S]methionine; lane 2, flow-through, HSV-2; Lane 3, KSCN fraction, HSV-2; lane 4, corresponding KSCN fraction from HSV-1–infected cells.

[^{35}S]methionine-labeled, purified gD-1 and gD-2 were also trypsinized directly and chromatographed on Chromabeads P. The profiles obtained for the 128K molecule (Fig. 2A), gD-1 isolated from the gel (Fig. 2B), and purified gD-1 (Fig. 2C) were virtually identical. The results indicate that there were no contaminating methionine peptides present in purified gD-1. The 128K molecule could have represented a dimer of gD-1 that was concentrated by purification, or it could have been an artifact generated during purification. The tryptic peptide profiles obtained for purified gD-2 (Fig. 3A) and gD-2 isolated from the gel (Fig. 3B) were virtually identical to each other. We conclude that gD-1 and gD-2 are both highly purified by immunoadsorbent chromatography.

Quantitation of gD activity. An antibody-binding assay (which made use of HD-1 immunoglobulin G was utilized to screen the cytoplasmic, flow-through, and KSCN fractions for gD-1 and gD-2 activities. Table 1 shows that the purification procedure resulted in a 421-fold increase in gD-1 activity and a 198-fold increase in gD-2 activity. The data in Table 1 emphasize the high yields (150 μg of gD-1; 82 μg of gD-2) and specific activities of both glycoproteins. The purification procedure resulted in a loss of approximately 50% of the antigenic activity of the HD-1 epitope of both gD-1 and gD-2 (Table 1).

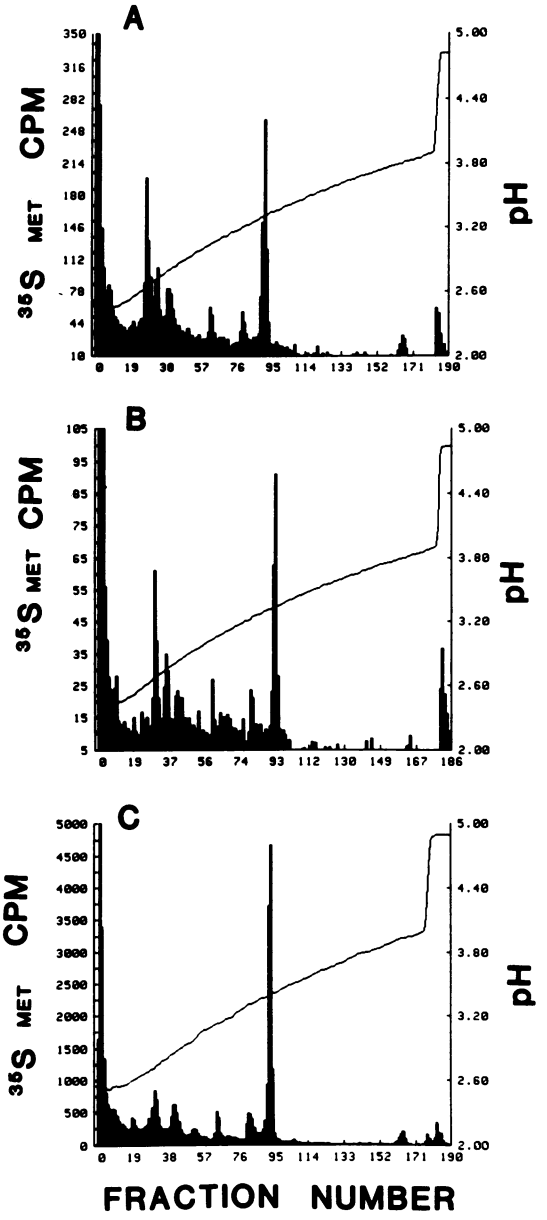

FIG. 2. Tryptic peptide analysis of [^{35}S]methionine-labeled gD-1 by cation-exchange chromatography. A, gD-1 was isolated from the 52K-to-60K region of a 10% sodium dodecyl sulfate-polyacrylamide gel (9, 20), dissolved in 2% periodic acid (10), and precipitated with 25% trichloroacetic acid. Procedures for oxidation, trypsinization, and chromatography on Chromabeads P have been described previously (5, 8, 9, 22). B, The 128K polypeptide was isolated from a 10% sodium dodecyl sulfate-polyacrylamide gel, prepared for trypsinization as described for gD-1 isolated from the gel, and chromatographed on Chromabeads P. C, Purified gD-1 isolated from the immunoadsorbent was precipitated directly with 25% trichloroacetic acid, trypsinized, and finally chromatographed on Chromabeads P.

Preliminary evidence indicates that HD-1 recognizes a conformational determinant of gD (J. Matthews, G. H. Cohen, and R. J. Eisenberg, unpublished data). Thus, the loss of activity may have been due to changes in the conformation of gD, possibly as a result of elution with the chaotropic agent KSCN (12).

Immunological activities of gD-1 and gD-2. (i) Serum-blocking capacity. Each preparation of gD was tested for serum-blocking capacity (19) against anti-CP-1 serum. For both glycoproteins, approximately 30 to 40 ng of gD was sufficient to reduce (by 50%) the neutralizing capacity of a fixed dilution of anti-CP-1 serum against both HSV-1 and HSV-2.

(ii) Immunogenicity. Both gD-1 and gD-2 were able to stimulate production of high titers of type-common neutralizing antibody in CAF/1 mice. The 50% neutralization titers (4) ranged from 512 to 2,048 for anti-gD-1 and from 192 to 1,536 for anti-gD-2. As expected, the titers for each antiserum were consistently higher against the homologous virus. However, all of the sera exhibited significant cross-neutralizing activity against the heterologous virus. Each of the antisera immunoprecipitated only gD from cytoplasmic extracts of HSV-1- or HSV-2-infected cells. This is further evidence of the purity of gD-1 and gD-2.

Amino acid composition of gD-1 and gD-2. The overall amino acid composition of gD-1 is similar but not identical to that of gD-2 (Table 2). This finding agrees with the previous prediction, which was based on tryptic peptide analysis (9). None of the amino acids was present in unusually high or low amounts in either glycoprotein, as compared with other membrane-associated glycoproteins (1, 11). The total numbers of acidic and basic amino acids were nearly the same in both glycoproteins. Thus, the overall charges of the precursor forms of both glycoproteins may be nearly the same. (Isoelectric focusing experiments indicate that pgD-1 has a pI of approximately 7.45 [5]. Preliminary experiments indicate that the pI of pgD-2 [7.12] is somewhat more acidic than that of pgD-1 [D. Long, G. H. Cohen, and R. J. Eisenberg, unpublished data].) The proportion of polar amino acids, the number of cysteine residues, and the number of methionine residues were very similar in the two glycoproteins. A few differences are also notable. For example, gD-2 contained more serine residues and fewer proline residues than did gD-1. As previously predicted from tryptic peptide analysis (9), gD-1 contained more arginine residues but fewer lysines than did gD-2. The differences in amino acid composition between gD-1 and gD-2 might not reflect large differences in the genes coding for those proteins. As previously noted (9), a single base change could change a

TABLE 1. Purification of gD-1 and gD-2 by immunoadsorbent chromatography[a]

Fraction	Determination					
	Total protein (mg)[b]	Total U of gD activity[c]	Sp act of gD (U/mg of protein)	Increase in sp act	Total amt of active gD (mg)	Recovery of gD activity (%)
Cytoplasm						
gD-1	180	1,714	9.5	1	0.205	100
gD-2	100	1,364	13.6	1	0.270	100
Flow-through						
gD-1	176	127	0.72	0.075	0.015	7.4
gD-2	99	129	1.3	1.3	0.025	7.5
KSCN						
gD-1	0.150	600	4,000	421	0.072	35
gD-2	0.082	222	2,700	198	0.044	16

[a] Conditions for preparation of the anti–HD-1 Sepharose immunoadsorbent and cytoplasmic extracts are described in the legend to Fig. 1.

[b] Determined by the method of Lowry et al. (15), as modified by Dulley and Grieve (7).

[c] Determined from a quantitative radioimmunoprecipitation assay. Increasing amounts of anti–HD-1 IgG were added to a fixed amount of radioactively labeled, purified gD-1 or gD-2. The mixtures were incubated for 20 min at 37°C, and *Staphylococcus aureus* was added to collect the immune complexes (14, 18). The complex was washed and suspended in sodium dodecyl sulfate disrupting buffer. Duplicate portions of each sample were counted in a scintillation counter, and the rest of the sample was analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis to ensure that all of the radioactivity bound by HD-1 was associated with gD. The amount of radioactivity bound was then expressed as nanograms of gD protein, based on the amount of trichloroacetic acid-precipitable radioactivity and protein (7, 15) in the KSCN sample. The amount of gD-1 or gD-2 bound to anti–HD-1 IgG was directly proportional to the concentrations of both antigen and antibody over a range of 25 to 200 ng of gD-1 or gD-2 and 0.1 to 1.0 μg of HD-1 IgG. A unit is defined as nanograms of gD bound/micrograms of HD-1 IgG. For gD-1, 1 U = 120 ng; for gD-2, 1 U = 198 ng.

TABLE 2. Amino acid compositions of gD-1 and gD-2[a]

Amino acid	No. of residues/molecule[b]	
	gD-1	gD-2
Aspartic acid	40	35
Threonine	24	23
Serine	46	62
Glutamic acid	53	59
Proline	35	27
Glycine	47	51
Alanine	37	44
Valine	23	20
Methionine	5	6
Isoleucine	23	19
Leucine	38	32
Tyrosine	15	7
Phenylalanine	11	5
Histidine	8	11
Lysine	16	22
Arginine	22	16
Cysteine	12	11
Tryptophan	ND[c]	ND

[a] Samples of gD-1 and gD-2 were dialyzed extensively against water, brought to 6 M HCl, and heated in vacuo at 110°C for 24, 48 and 72 h. Amino acids were quantitated on a Dionex D500 Amino Acid Analyzer. The values for serine and threonine were calculated by extrapolation to zero time. The amounts of isoleucine, leucine, and valine were calculated on the basis of 48 and 72-h hydrolyses. Cysteine was determined after performic acid oxidation (13).

lysine codon to an arginine codon. A single base change could also convert a proline codon to a serine codon (e.g., CCG → UCG or CCC → UCC). However, differences in proline and serine contents might have a somewhat greater effect on the relative conformations and stabilities of the two proteins. Thus, some of the structural and antigenic differences between gD-1 and gD-2 could result from small changes in the nucleotide sequence. Further structural studies of the purified proteins and their nucleic acid templates will be required to understand the antigenic similarities of and differences between gD-1 and gD-2.

[b] For gD-1, the total number of amino acids was assumed to be 455 (average molecular weight per amino acid, 110). For gD-2, the total number of amino acids was assumed to be 450 (average molecular weight per amino acid, 110). The molecular weight of gD-1 minus carbohydrate was assumed to be 50,000, based on results with tunicamycin (18). The molecular weight of gD-2 minus carbohydrate was assumed to be 49,500, based on similar experiments with tunicamycin (Matthews et al., unpublished data).

[c] ND, Not determined.

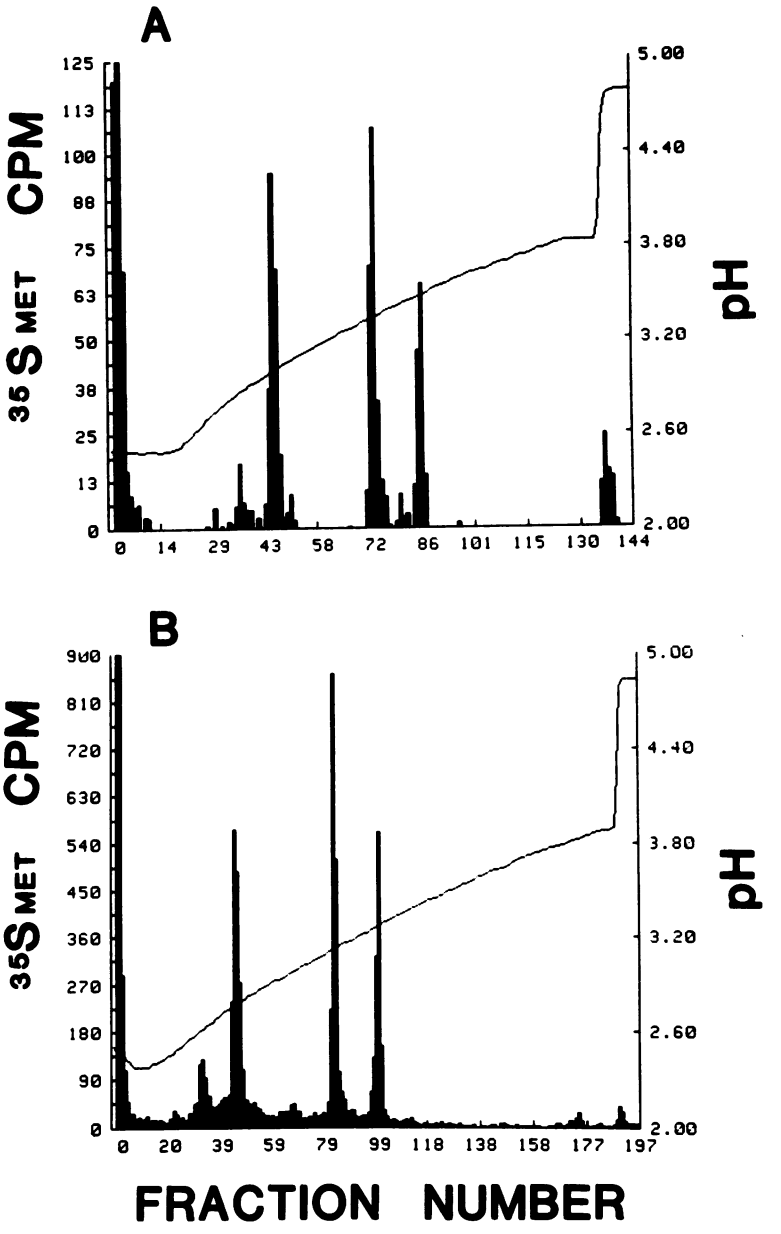

FIG. 3. Tryptic peptide analysis of [^{35}S]methionine-labeled gD-2 by cation-exchange chromatography. A, gD-2 isolated from a 10% sodium dodecyl sulfate-polyacrylamide gel (see legend to Fig. 2 for details of isolation). B, Purified gD-2 (KSCN fraction from immunoadsorbent; see legend to Fig. 2 for details of isolation and trypsinization).

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