

# Primate Cytomegalovirus Glycoproteins: Lectin-Binding Properties and Sensitivities to Glycosidases

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The lectin-binding properties and glycosidase sensitivities of the virion glycoproteins of primate cytomegaloviruses (CMVs) were examined. Three simian CMV (SCMV) strains, including Colburn, and four human CMV (HCMV) strains were compared. Their proteins were separated in denaturing polyacrylamide gels and electrotransferred onto nitrocellulose, and the glycosylated species were visualized with iodinated concanavalin A or wheat germ agglutinin (WGA). Virions of both HCMV and SCMV strains contained six principal and several minor lectin-reactive bands. Neuraminidase treatment abolished WGA binding and reduced the charge and charge heterogeneity of the SCMV (i.e., Colburn) virion glycoproteins and had a similar, although less dramatic, effect on those of HCMV. The specificities of concanavalin A and WGA in these assays were evaluated with endo- $\beta$ -N-acetylglucosaminidase H and endo- $\beta$ -N-acetylglucosaminidase F, and a combination of lectins and glycosidases was used to demonstrate that many of the primate CMV glycoproteins contain both high-mannose and complex, N-linked oligosaccharides. Results suggest that the HCMV virion glycoproteins are more extensively glycosylated or have more completely processed carbohydrate side chains, or both, than their SCMV counterparts.

The glycoproteins of enveloped viruses are of particular biological interest since they typically constitute the antigens involved in virus neutralization, mediate entry, and may play a role in the release of the virion from the host cell (38). An important means of identifying this set of proteins has been by selective biosynthetic labeling with radioactive sugars (e.g., [ $^3$ H]glucosamine) followed by electrophoretic separation in sodium dodecyl sulfate (SDS)-containing polyacrylamide gels.

This approach, applied to the virion glycoproteins of strain Colburn cytomegalovirus (CMV), a simian or simian CMV-like (SCMV) strain, revealed eight well-resolved [ $^3$ H]glucosamine-labeled bands ranging in molecular weight from approximately 163,000 to 27,000 and ranging in charge from approximately neutral to acidic (12). In contrast, the virion glycoproteins of human strains of CMV (HCMV) showed a complex electrophoretic profile composed of two diffuse, comparatively well-labeled bands and several minor ones (3, 12, 14, 20, 32). This disperse appearance made it difficult to assign accurate molecular weights or even to determine the exact number of virion glycoproteins. Two-dimensional (charge-size) separations helped to resolve the complexity of the HCMV pattern. The two broad bands (gp145 and gp57) were shown to be more acidic than the other virion proteins, and the presence of a less intensely labeled 62-kilodalton (kDa) glycoprotein, which is comprised of several discrete charge isomers (gp62), was revealed (12, 14).

The greater heterogeneity of HCMV glycoproteins, compared with those of strain Colburn, may reflect important differences between these strains in glycoprotein processing or associated maturational events (e.g., envelopment and egress from the cell). Therefore, further study of their oligosaccharides is of interest. Since the amount of material available was insufficient for direct biochemical analyses, indirect techniques have been applied. This report presents an evaluation of both HCMV and SCMV virion glycopro-

teins based on lectin binding, glycosidase sensitivity, and peptide comparisons. The results presented here are in good agreement with earlier findings based on labeling with radioactive sugars (3, 12, 14, 20, 32), are consistent with initial lectin gel overlay (27) and glycosidase (7) experiments, and provide new information about the structure and linkage of the oligosaccharides attached to primate CMV glycoproteins.

(Part of this work was presented at the 9th and 10th International Herpesvirus Workshops in Seattle, Wash. [1984] and Ann Arbor, Mich. [1985].)

## MATERIALS AND METHODS

**Cells and viruses.** Procedures used in preparing, maintaining, and infecting human foreskin fibroblast cell cultures have been described before (11). Cells were grown in Dulbecco modified essential medium (no. 430-2100; GIBCO Laboratories, Grand Island, N.Y.) containing 10% fetal calf serum (HyClone Sterile Systems, Logan, Utah). Many of the experiments described here were done with strain Colburn CMV. Other CMV strains used were of both human (HCMV strains AD169, Davis, Towne, and 751) and simian (SCMV strains CSG, SA6, and AGSGV) origin. The sources and additional biochemical details of these isolates are reported elsewhere (12). The SCMV isolate AGSGV was kindly provided by Richard Heberling and is thought to be the same as CSG since all of the tested biological and biochemical properties of the two strains are the same.

**Biosynthetic radiolabeling of virus particles.** Virus-infected cell cultures were labeled, beginning 2 days after infection, with [ $^{35}$ S]methionine (no. SJ.204, 10  $\mu$ Ci/ml), [ $^3$ H]glucosamine (no. TRK.398, 50  $\mu$ Ci/ml), or [ $^3$ H]fucose (no. TRK.477, 50  $\mu$ Ci/ml), all from Amersham Corp., Arlington Heights, Ill., and each in complete maintenance medium. Four days later, virions were recovered from the cultures as described below.

**Virus isolation.** Virions were recovered from the maintenance medium essentially as described before (18), but using sucrose gradients (15 to 50% [wt/vol] in 40 mM

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phosphate buffer–150 mM NaCl [pH 7.4]) instead of tartrate-glycerol gradients, and were subjected to centrifugation for 20 min at 40,000 rpm and 4°C in an SW41 rotor (Beckman Instruments, Inc., Palo Alto, Calif.) or for 40 min at 25,000 rpm and 4°C in a Sorvall AH627 rotor (Du Pont Co., Wilmington, Del.). The virion band was collected and combined with an equal volume of the gradient buffer, pelleted by centrifugation for 2.5 h at 35,000 rpm and 4°C in a Sorvall AH650 rotor, and stored at –80°C after solubilization in SDS-polyacrylamide gel electrophoresis (PAGE) sample buffer (see below) or resuspension in an appropriate buffer.

**In vitro radioiodination of virus particles.** After isolation as described above, virus pellets were suspended in 40 mM sodium phosphate buffer (pH 8.5) and radiolabeled with Na<sup>125</sup>I (no. IMS.300, Amersham) for 10 min at room temperature by using 5 µg of chloramine T (BDH, Poole, England) per 50-µl reaction volume. The reaction was terminated by adding 20 µl of a tyrosine solution (0.4 mg/ml of H<sub>2</sub>O), and the buffer was exchanged with 40 mM sodium phosphate buffer–150 mM NaCl (pH 7.4) by using centrifugal microconcentrators (Centricon-10; Amicon Corp., Danvers, Mass.). Preparations were solubilized and subjected to SDS-PAGE, as described below, or stored at –80°C until used.

**PAGE.** SDS-PAGE was done essentially as described by Laemmli (22) in gels prepared with an acrylamide/methylene-bis-acrylamide ratio of 38:1. Unless stated otherwise, resolving gels were 10% acrylamide, stacking gels were 3.6% acrylamide, and both gel and electrode buffer contained SDS (no. 28365; Pierce Chemical Co., Rockford, Ill.). Samples were solubilized by heating for 3 min at 100°C in SDS-PAGE sample buffer (2% SDS, 10% beta-mercaptoethanol, 10% glycerol, 0.005% bromophenol blue, 50 mM Tris [pH 7.0]). After electrophoresis, gels containing proteins to be used subsequently for treatment with endoglycosidases or for peptide analysis (see below) were rinsed for 30 to 60 min in water containing approximately 25 mg of mixed bed resin per ml (no. AG501-X8; Bio-Rad Laboratories, Richmond, Calif.) to remove excess Tris, glycine, and SDS. Two-dimensional (charge-size) separations of proteins in denaturing polyacrylamide gels (two-dimensional PAGE) were done as described elsewhere (9, 17). Proteins were visualized by staining the gel with Coomassie brilliant blue (6) or ammoniacal silver (39), by fluorography after diphenyloxazole treatment (2, 23), or by using calcium tungstate intensifying screens (33).

**Protein transfer to nitrocellulose and analyses using radiolabeled lectins.** Virion proteins were electrophoretically transferred (2 h, 12 V) from SDS-containing, 10% polyacrylamide gels onto nitrocellulose by the method of Towbin et al. (35). Transfer of high-molecular-weight species was facilitated by incorporating pronase (no. 537088; Calbiochem-Behring, La Jolla, Calif.) into the procedure, as described before (10). After transfer, the resulting nitrocellulose replicas were quenched in phosphate-buffered saline (137 mM NaCl, 2.7 mM KCl, 1.5 mM KH<sub>2</sub>PO<sub>4</sub>, 8 mM Na<sub>2</sub>HPO<sub>4</sub>) (PBS) containing 2% polyvinylpyrrolidone-40 (Sigma Chemical Co., St. Louis, Mo.) (PBS–PVP-40) at room temperature for 30 min or overnight at 4°C (1).

Quenched replicas were incubated with radioiodinated concanavalin A (<sup>125</sup>I-ConA) prepared in ConA buffer (5 mM Tris, 150 mM NaCl, 10 mM CaCl<sub>2</sub>, 1 mM MgCl<sub>2</sub> [pH 7.0]) containing 0.5% hemoglobin (no. H-2500, Sigma), for 3 h at room temperature with continuous agitation. Replicas to be reacted with radioiodinated wheat germ agglutinin (<sup>125</sup>I-WGA) were first treated to enhance sialic acid recognition by the technique of Bartles and Hubbard (1). They were then

incubated with <sup>125</sup>I-WGA (diluted in PBS–PVP-40) for 3 h at 4°C with continuous agitation, rinsed for 30 min in six changes of PBS, dried, and exposed to Kodak XAR-5 film at –70°C with calcium tungstate intensifying screens (33).

**Iodinated lectins.** ConA (no. L-1104) and WGA (no. L-2101), both from E-Y Laboratories, San Mateo, Calif., were rehydrated in ConA buffer and PBS, respectively, to a concentration of 10 mg/ml and radiolabeled with Na<sup>125</sup>I (no. IMS.300; Amersham) and chloramine T (0.5 mg/ml of ConA buffer or PBS) for 60 s at room temperature. WGA was radiolabeled in the presence of 170 mM *N*-acetylglucosamine to protect binding-site tyrosine residues (1). Reactions were stopped by adding 40 µl of a tyrosine solution (0.4 mg/ml of H<sub>2</sub>O) to the 200-µl reaction mixture. Iodinated lectins were concentrated and the buffer was exchanged by using centrifugal microconcentrators (Centricon-30, Amicon) and then dialyzed for 12 h at 4°C against PBS or ConA buffer. Labeled WGA was stored at 4°C in PBS–PVP-40 to prevent freeze-thaw inactivation; labeled ConA was stored at –20°C in ConA buffer containing 0.5% hemoglobin.

**Treatment of virus particles with neuraminidase.** [<sup>35</sup>S] methionine-labeled virus particles were incubated with neuraminidase (0.33 U/ml, derived from *Vibrio cholerae*; Calbiochem-Behring) in buffer (50 mM sodium acetate, 154 mM NaCl, 4 mM CaCl<sub>2</sub> [pH 5.5]) for 30 min at 37°C. Samples were dialyzed and concentrated to dryness in collodion bags (no. UH 100/25; Schleicher & Schuell, Inc., Keene, N.H.) against 50 mM Tris [pH 7.0] and were then solubilized in SDS-PAGE sample buffer.

**Treatment of glycoproteins with endoglycosidases.** Polyacrylamide gel slices containing radioiodinated glycoproteins were rehydrated in endo F buffer (100 mM sodium phosphate buffer [pH 8.6], 20 mM EDTA, 0.1% SDS, 0.1% Nonidet P-40, 50 mM 2-mercaptoethanol) or endo H buffer (50 mM sodium acetate [pH 6.0], 0.1% SDS) and then forced through wire screens (e.g., CX-200; Small Parts, Inc., Miami, Fla.) with enough additional buffer to produce gel slurries. The slurries were incubated with endo H (endo-β-*N*-acetylglucosaminidase H) (Health Research, Inc., Albany, N.Y.) or endo F (endo-β-*N*-acetylglucosaminidase F) (no. NEE150; New England Nuclear Corp., Boston, Mass.) at 37°C for 14 h, solubilized, and analyzed by SDS-PAGE.

**Protein comparison by partial proteolysis.** [<sup>35</sup>S]methionine-labeled virions were solubilized and subjected to SDS-PAGE as described above. The lane of interest was cut from the first gel, applied to the top of a second gel, and subjected to partial proteolysis with 10 µg of *Staphylococcus aureus* V-8 protease per ml (no. 39-900; Miles Scientific, Div. Miles Laboratories, Inc., Naperville, Ill.) essentially as described by Cleveland et al. (4). After electrophoresis, the second gel was stained and prepared for fluorography.

**Cyanogen bromide peptide mapping.** Polyacrylamide gel slices containing radioiodinated viral glycoproteins were rehydrated and exposed to cyanogen bromide (Aldrich Chemical Co., Inc., Milwaukee, Wis.) for 1 h, as described by Pepinsky (28). After excess cyanogen bromide was removed by multiple washes, the gel slices were soaked in a small volume of SDS-PAGE sample buffer and then forced through a wire screen, as described above. Resulting cleavage products in the gel slurries were analyzed by SDS-PAGE in *N,N'*-diallyltartardiamide–cross-linked, 16% polyacrylamide gels.

## RESULTS

**Identification of nitrocellulose-bound CMV glycoproteins with radiolabeled lectins.** The efficiency of transferring CMV

glycoproteins from polyacrylamide gels onto nitrocellulose was tested. Radiolabeled virions of strain Colburn CMV were solubilized and subjected to SDS-PAGE, and the separated proteins were electrotransferred to nitrocellulose. Figure 1 shows that the pattern of [ $^3\text{H}$ ]glucosamine-labeled glycoproteins transferred onto the nitrocellulose was qualitatively the same as that in the gel (compare lanes b and c). Densitometric measurements of these two lanes, however, indicated that the relative intensity of the nitrocellulose-bound gp163 band was weaker than that in the gel. Evidence presented elsewhere (13) indicates that this is due to the comparatively poor binding of its glycopeptides to nitrocellulose. This figure also shows the pattern of [ $^3\text{H}$ ]fucose-labeled Colburn virion glycoproteins (lane d) and demonstrates that all of the [ $^3\text{H}$ ]glucosamine-labeled bands, except gp61 and possibly gp27, were also labeled with [ $^3\text{H}$ ]fucose.

The lectin-binding properties of the nitrocellulose-bound virion proteins were then examined as follows. Metabolically labeled ( $^{35}\text{S}$ ]methionine) and nonlabeled Colburn virions were isolated from infected cultures, solubilized, and subjected to SDS-PAGE. The proteins were electrotransferred onto nitrocellulose, and the resulting replicas were probed with  $^{125}\text{I}$ -ConA to detect mannose-containing glycoproteins (15, 19, 21) or with  $^{125}\text{I}$ -WGA to detect sialic acid- and *N*-acetylglucosamine-containing glycoproteins (25, 26, 40). WGA incubation conditions were adjusted for enhanced sialic acid binding (1). Nonlabeled strain 751 HCMV virion glycoproteins were analyzed in parallel.

Radiographic and fluorographic images prepared from the resulting replicas (Fig. 2) showed that most of the Colburn and HCMV bands that had been provisionally designated glycoproteins on the basis of selective biosynthetic labeling with [ $^3\text{H}$ ]glucosamine (12, 14), were also selectively labeled when transferred to nitrocellulose and incubated with iodinated lectins. Results of these analyses and of later lectin-binding assays of endoglycosidase-treated material (see Fig. 10B) indicate that Colburn gp163, gp119, gp100, and gp65 and HCMV gp145 and gp62 all contain high-mannose N-linked oligosaccharides (i.e., bind ConA) and sialic acid (i.e., bind WGA). The reactivity of WGA with Colburn gp65 and HCMV gp62 was comparatively weak, and the presence of sialic acid on these proteins is better demonstrated by the neuraminidase experiments (see Fig. 5 and 6). By these criteria, Colburn gp61 and gp49 (see arrow, lane c, Fig. 2) and HCMV gp130 contained high-mannose side chains but not sialic acid. In contrast, Colburn gp59 and its HCMV counterpart gp57 contained sialic acid but did not react with ConA. Three Colburn bands that were labeled with [ $^3\text{H}$ ]glucosamine were not recognized by either lectin in this experiment (i.e., gp39, gp36, and gp27); however, gp39 was found to react with ConA after exposure to V-8 protease (e.g., see Fig. 8). This finding is unexplained.

Since WGA is reactive with *N*-acetylglucosamine as well as with sialic acid (1, 25, 26, 40), the following experiment was done to determine which carbohydrates were being recognized on the CMV glycoproteins. Colburn virions were solubilized and subjected to SDS-PAGE. The proteins were then transferred onto nitrocellulose and reacted with  $^{125}\text{I}$ -WGA after the following treatments: (i) no enhancement, (ii) enhancement with metaperiodate and oxidized aniline (i.e., brown color), (iii) enhancement with metaperiodate and fresh aniline (i.e., faint yellow color), and (iv) incubation with neuraminidase before enhancement with metaperiodate and fresh aniline. A 44-kDa glycoprotein (orosomucoid) containing both glucosamine and terminal sialic acid residues was used as a control. Results of the experiment show

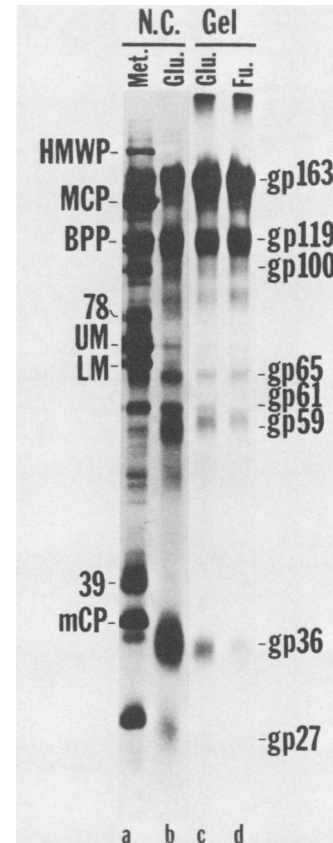


FIG. 1. Glycoproteins of Colburn CMV on nitrocellulose (N.C.) or in a polyacrylamide gel (Gel). Virions were recovered from the media of Colburn CMV-infected cells radiolabeled with [ $^{35}\text{S}$ ]methionine (Met.), [ $^3\text{H}$ ]glucosamine (Glu.), or [ $^3\text{H}$ ]fucose (Fu.) at concentrations of 10, 50, and 50  $\mu\text{Ci/ml}$  of complete medium, respectively. The virion preparations were solubilized and subjected to SDS-PAGE. After electrophoresis, a section of the gel (panel labeled Gel) containing [ $^3\text{H}$ ]glucosamine- and [ $^3\text{H}$ ]fucose-labeled proteins was processed for fluorography. The proteins in another section of the gel (panel labeled N.C.), containing [ $^3\text{H}$ ]glucosamine- and [ $^{35}\text{S}$ ]methionine-labeled preparations, were electrotransferred onto nitrocellulose. The nitrocellulose strip was then sprayed with  $\text{En}^3\text{Hance}$  (New England Nuclear) and exposed to Kodak XAR film at  $-70^\circ\text{C}$ . Shown here is a photograph of the resulting fluorographic images. Numbers in the left- and right-hand margins indicate the molecular weight (in thousands) of the corresponding band. Abbreviations are as follows: HMWP, high-molecular-weight protein; MCP, major capsid protein; BPP, basic phosphoprotein; UM and LM, upper and lower matrix proteins, respectively; mCP, minor capsid protein; and gp, glycoprotein.

that orosomucoid reacted as was described by Bartles and Hubbard (1) (Fig. 3). Specifically, the binding of WGA was enhanced at least 100-fold after the metaperiodate-aniline treatment. The more highly oxidized aniline enhanced binding more than did fresh aniline. The virion proteins behaved differently. While WGA binding to gp59 and gp65 was markedly enhanced (about 60-fold) by the aniline pretreatment, that of gp163 was not enhanced, and that of gp119 was increased by only sixfold. When the nitrocellulose-bound glycoproteins were treated with neuraminidase, WGA binding was reduced by more than 98%.

For purposes of comparison, the glycoproteins of other HCMV and SCMV isolates were examined by the nitrocellulose-lectin procedure. Virions were prepared from cells infected with HCMVs Towne, AD169, Davis, and 751 and

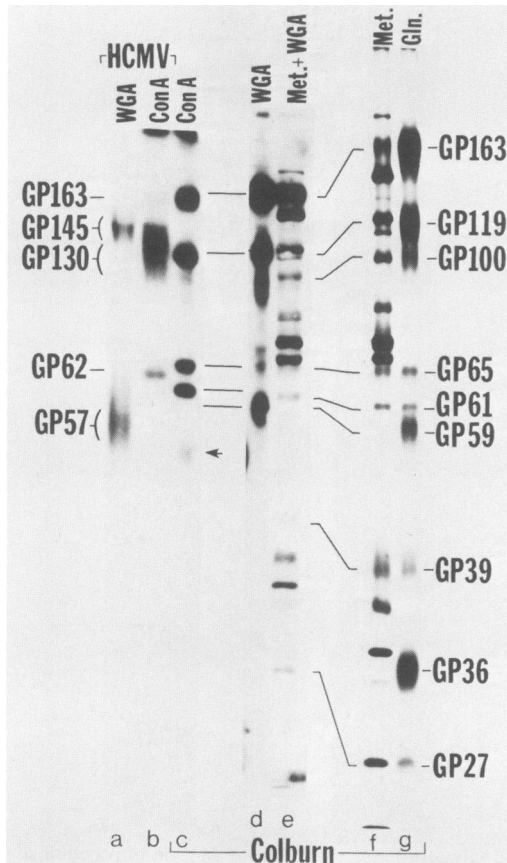


FIG. 2. Lectin-binding analysis of HCMV and Colburn virion glycoproteins. Biosynthetically radiolabeled or nonlabeled virion proteins were separated by SDS-PAGE, electrotransferred onto nitrocellulose, probed with  $^{125}\text{I}$ -WGA or  $^{125}\text{I}$ -ConA, and analyzed by fluorography. Shown here is a composite of fluorograms prepared from three experiments: (i) lanes f and g show biosynthetically labeled Colburn virion proteins separated by SDS-PAGE and visualized in the gel (7.5% polyacrylamide); (ii) lanes d and e show nonlabeled and biosynthetically labeled Colburn virion proteins, respectively, after transfer from a 10% polyacrylamide gel onto nitrocellulose and incubation with  $^{125}\text{I}$ -WGA; and (iii) lanes a, b, and c show nonlabeled HCMV (lanes a and b) and Colburn (lane c) virion proteins after transfer from a 10% polyacrylamide gel onto nitrocellulose and incubation with  $^{125}\text{I}$ -WGA or  $^{125}\text{I}$ -ConA, as indicated. Abbreviations: Met., [ $^{35}\text{S}$ ]methionine labeled; Gln, [ $^3\text{H}$ ]glucosamine labeled. Glycoproteins (GP) are designated by molecular weight (in thousands). Arrow denotes position of Colburn gp49.

SCMV's Colburn, SA6, CSG, and AGSGV. Their proteins were solubilized, separated by SDS-PAGE, transferred onto nitrocellulose, and reacted with  $^{125}\text{I}$ -ConA or  $^{125}\text{I}$ -WGA. Results of this experiment (Fig. 4) showed that the lectin-binding properties of counterpart Towne, AD169, Davis, and 751 glycoproteins were qualitatively similar; however, as reported before (14, 27), there were differences in the mobility of the gp62 band among these strains. Further, the WGA-binding pattern for the HCMV strains was significantly more diffuse than the corresponding ConA-binding pattern. Six principal HCMV bands were detected with the iodinated lectins. ConA bound to bands designated gp163, gp145, gp130, and gp62; WGA bound to bands designated gp145, gp70, and gp57. At least three of these glycoproteins (gp145, gp62, and gp57) have previously been shown to radiolabel with [ $^3\text{H}$ ]glucosamine (12, 14). ConA binding at

the upper matrix and lower matrix positions (HCMVs Davis and 751) is attributed to nonspecific binding by these two matrix proteins. It is possible, however, that a fraction of these proteins may be glycosylated or that these bands also contain low-abundance glycoproteins. The Towne HCMV preparation used in this study was biosynthetically labeled with [ $^{35}\text{S}$ ]methionine to provide internal markers (i.e., major capsid, upper matrix, lower matrix, and minor capsid proteins).

There was more variability among the counterpart glycoproteins of the SCMV strains. All showed six predominant bands that reacted with the lectins. In strain Colburn, gp163, gp119, gp100, gp65, gp61, and gp49 reacted with ConA; gp163, gp119, gp100, gp65, and gp59 reacted with WGA. Compared with the Colburn bands, the SA6 and CSG counterparts showed both qualitative and quantitative differ-

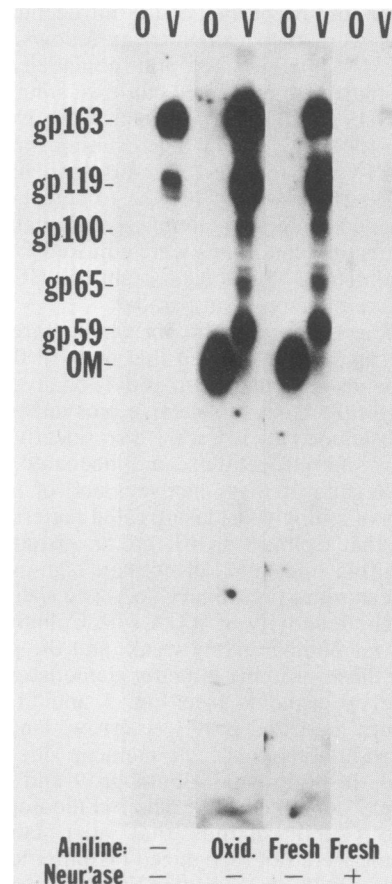


FIG. 3. Enhanced WGA binding to Colburn virion glycoproteins. Four sets of nonlabeled Colburn virion proteins (lanes marked V) and orosomucoid (lanes marked O) were subjected to SDS-PAGE, electrotransferred onto nitrocellulose, and further treated with combinations of metaperiodate-aniline-cyanoborohydride, neuraminidase, and  $^{125}\text{I}$ -WGA, as described in the text. Neuraminidase treatment was done by incubating the PBS-PVP-40-quenched strip of nitrocellulose in a solution (50 mM sodium acetate, 154 mM NaCl, 4 mM  $\text{CaCl}_2$  [pH 5.5]) containing 0.13 U of *V. cholerae* neuraminidase per ml for 12 h at 22°C. After incubation with the enzyme, the nitrocellulose strip was again quenched with PBS-PVP-40 and then processed for enhanced detection of sialic acid (1). Shown here are fluorographic images of the resulting nitrocellulose strips. Numbers in the left-hand margin indicate the molecular weight (in thousands) of the corresponding band. Other abbreviations: gp, glycoprotein; OM, orosomucoid (no. 82-313-1; Miles Laboratories, Inc., Elkhart, Ind.); Neur.'ase, neuraminidase; Oxid., oxidized.

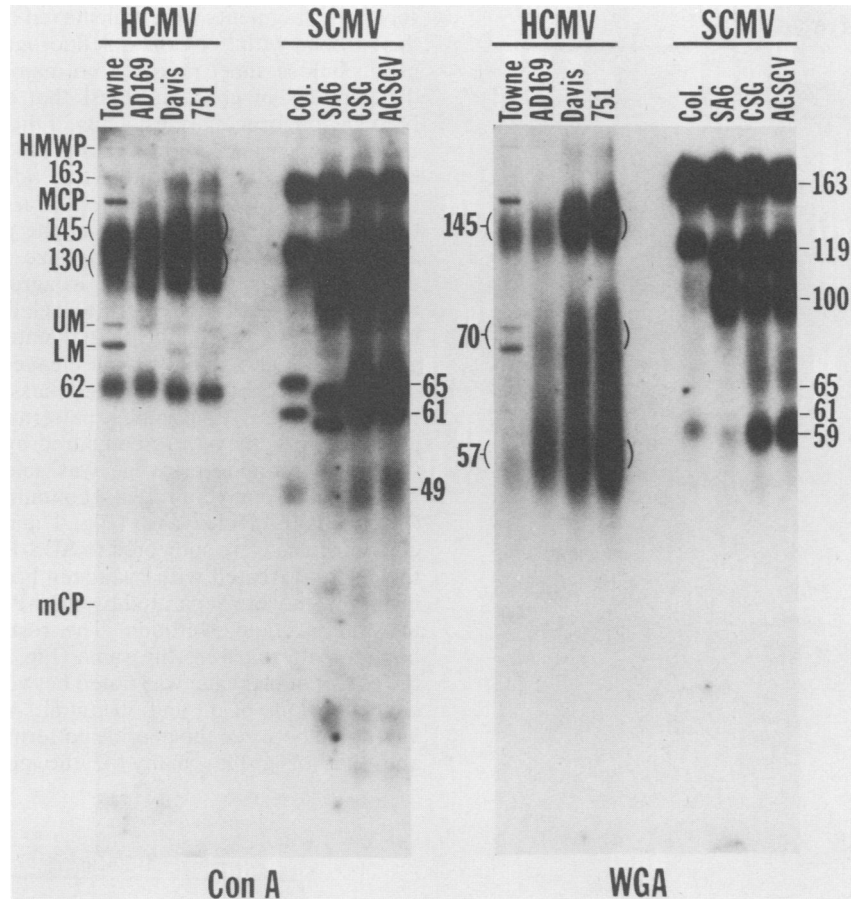


FIG. 4. Lectin-binding analysis of HCMV and SCMV virion glycoproteins. Virions were recovered from the culture media of cells infected with the indicated HCMV and SCMV strains, concentrated by pelleting, subjected to SDS-PAGE, electrotransferred onto nitrocellulose, and incubated with iodinated WGA (panel marked WGA) or ConA (panel marked ConA). Comparable amounts of virions were present in each preparation. The estimated relative amount of major capsid protein (MCP) was AGSGV>SA6≥751>Davis>AD169>>Towne. Shown here is a photograph of fluorograms prepared from the resulting nitrocellulose sheets. Abbreviations are as in the legend to Fig. 1; numbers indicate the molecular weight (in thousands) of the corresponding band. The Towne preparation contained biosynthetically labeled (<sup>35</sup>S)methionine) proteins to serve as markers.

ences in lectin binding. These can be summarized as follows. (i) The apparent counterparts to gp119, as revealed by WGA binding, migrated faster. (ii) The SA6 counterparts to gp65 and gp61, as shown by ConA binding, both migrated faster. (iii) The CSG counterparts to gp119 and gp65, and to a lesser degree the SA6 counterpart to gp65, showed more heterogeneity in size when visualized with labeled ConA. (iv) The SA6 and CSG counterparts to gp119 were more intensely labeled by WGA and ConA than was the Colburn band, and the CSG gp59 counterpart was more intensely labeled by WGA than its corresponding band in either Colburn or SA6. The indistinguishable lectin-binding patterns of CSG and AGSGV are consistent with their other biological and biochemical similarities.

**Effect of desialylation on Colburn and HCMV glycoproteins.** The glycoproteins recognized most strongly by WGA have been shown by two-dimensional PAGE to be comparatively acidic in net charge and to exhibit multiple charge isomers (12, 14). To determine the extent to which sialic acid contributes to the overall charge of these glycoproteins, neuraminidase was used to remove terminal sialic acid residues from intact, [<sup>35</sup>S]methionine-labeled Colburn and HCMV strain 751 virions, as described in Materials and Methods. Results of this experiment (Fig. 5 and 6) and of others not shown (e.g., charge-size separations of glucos-

amine-labeled, neuraminidase-treated Colburn virion proteins) demonstrated the following. First, the four most acidic charge isomers of Colburn gp65 were apparently converted to one major and one minor spot located directly above the two gp61 spots. Second, gp119 and gp100 were apparently converted to a small number of less acidic spots immediately below and in the same charge range as the major capsid protein. Third, both charge species at the SDS-PAGE position of gp163 were affected. The highly acidic spot labeled gp163 was eliminated, and the unresolved zone at the basic side of the pattern was replaced by a tighter spot having a more basic charge and an 8% slower electrophoretic mobility. Fourth, nonglycosylated proteins, such as the major capsid protein, the matrix proteins, and the minor capsid protein, were unaffected by neuraminidase treatment. Finally, it was found that neuraminidase treatment reduced but did not eliminate the charge heterogeneity of HCMV gp62 (Fig. 6). Specifically, gp62 spots 2, 3, 4, and 5 were found to be closely spaced doublets in the nontreated preparation (Fig. 6, top panel). After treatment with neuraminidase, the acidic (left-hand) partner of each of these pairs was eliminated, as was spot 6 (Fig. 6, bottom panel). An apparent consequence of these decreases was a corresponding increase in the relative intensities of spot 1 and the right-hand partner of doublets 2, 3, and 4. Reference lines connecting



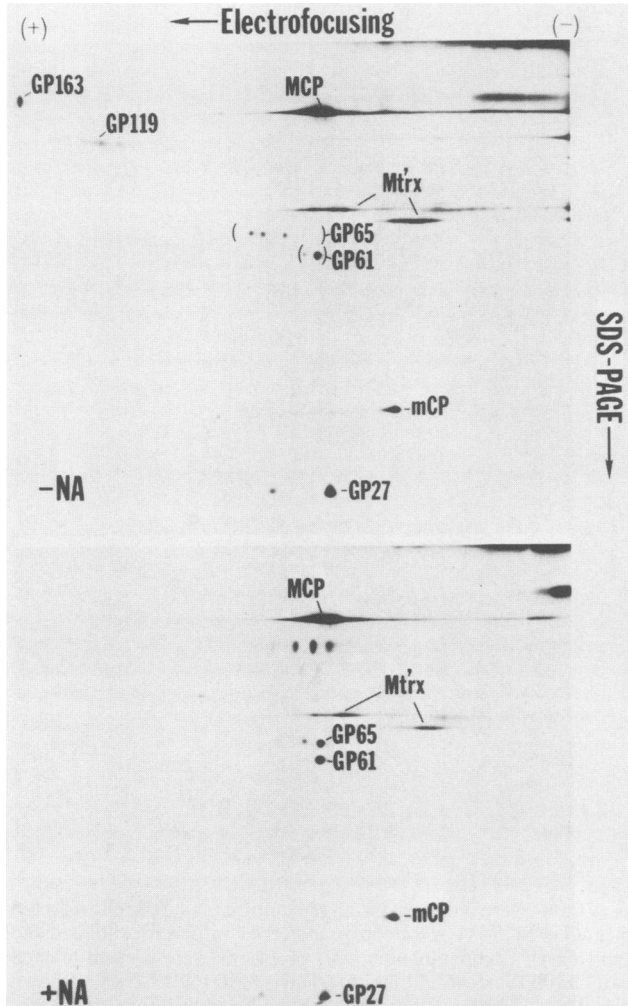


FIG. 5. Effect of desialylation on Colburn virion glycoproteins. [<sup>35</sup>S]methionine-labeled Colburn virions were incubated with (+NA) or without (-NA) neuraminidase, and then were solubilized, subjected to two-dimensional PAGE, and analyzed by fluorography. Abbreviations: NA, neuraminidase; M'rx, matrix proteins; MCP, major capsid protein; mCP, minor capsid protein. Electrofocusing was from right (cathode) to left (anode). Glycoproteins (GP) are designated by molecular weight (in thousands).

spots that were unaffected by the treatment were drawn to simplify the comparison.

**Peptide comparisons.** The relatedness of the Colburn virion glycoproteins was tested by partial proteolysis as follows. [<sup>35</sup>S]methionine-labeled Colburn virions were solubilized and subjected to SDS-PAGE. The gel lane was excised and applied to the top of a second gel, and partial proteolysis with *S. aureus* V-8 protease was done on the intact strip. A fluorogram prepared from the resulting gel shows that proteolytic cleavage of gp65 and gp61 produced indistinguishable peptide patterns (see arrows, Fig. 7), indicating that the two proteins share closely related amino acid sequences. Neither gp163, gp39, nor a band just above the 78-kDa protein (see arrow) was noticeably affected by the protease (i.e., relative migration the same in first and second gel).

The glycosylated peptides of gp65 and gp61 were also compared (Fig. 8). Virion proteins were resolved and subjected to partial proteolysis as described above, and the

separated fragments were transferred onto nitrocellulose and then probed with <sup>125</sup>I-ConA. A fluorogram prepared from the nitrocellulose sheet revealed no major differences between the peptides of gp65 and gp61 that contain high-mannose N-linked oligosaccharides. Thus, if the difference in mobility between gp65 and gp61 is related to the addition of more high-mannose carbohydrates, that addition is not reflected by new ConA-binding peptides. ConA-reactive proteolytic fragments were also detected at the position of gp119 and appeared to be about the same size as, but more discrete than, those of gp65 and gp61. In agreement with results of the preceding experiment, radiolabeled spots on the diagonal between the two gels indicate that material at the positions of gp163, gp87, and gp39 was not cleaved by V-8 protease.

To confirm the apparent relatedness of gp65 and gp61 and to obtain proteolytic fragment patterns for some of the other glycoproteins, they were compared by a second technique. Cyanogen bromide cleavage was selected since all of the major Colburn glycoproteins contain methionine residues (i.e., the site of cleavage) (Fig. 1 and 2). Iodinated virion glycoproteins were subjected to SDS-PAGE, sectioned from the gel, and treated with cyanogen bromide, and the resulting peptides were separated by SDS-PAGE, all as described in Materials and Methods. The resulting gp65 and gp61 peptide patterns were the same (Fig. 9); little difference in the extent of cleavage was noted between cyanogen bromide concentrations of 35 and 70 μg/ml. Although no similarity was noted between the peptide patterns of gp65 and gp61 and that of gp119, many of the gp65 and gp61 bands,

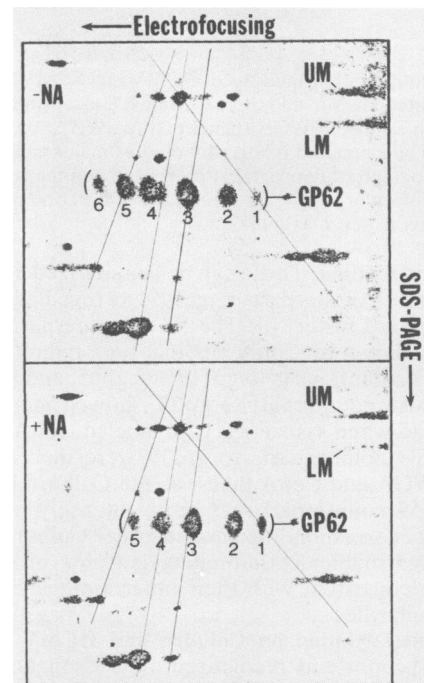


FIG. 6. Effect of desialylation on HCMV gp62. [<sup>35</sup>S]methionine-labeled HCMV strain 751 virions were incubated with (+NA) or without (-NA) neuraminidase and then were solubilized and separated by two-dimensional PAGE. Shown here are portions of the fluorographic images of the second-dimension gels, with reference lines drawn through spots whose positions did not change. All but one reference line pass through three reference spots in addition to one of the gp62 spots. Abbreviations: NA, neuraminidase; UM, upper matrix protein; LM, lower matrix protein.

including the noncleaved proteins (see arrowheads), comigrated with bands in the gp163 pattern (indicated).

**Effect of endo H and endo F on CMV glycoproteins.** Iodinated glycoproteins of strain Colburn virions were subjected to SDS-PAGE, sectioned from the gel, and treated with either endo H to remove high-mannose N-linked oligosaccharides (34) or endo F to remove both high-mannose and complex, N-linked oligosaccharides (5, 30). The procedure used is described in Materials and Methods. Analysis of the treated proteins by SDS-PAGE (Fig. 10A) showed the following. (i) Endo H and endo F each converted gp61 to a 56-kDa band. (ii) Endo H converted gp65 to a band that migrated close to the position of gp61; endo F converted gp65 to a 56-kDa band that comigrated with the endo H and endo F products of gp61. (iii) Endo H had only small effects on the electrophoretic mobilities of gp163 and gp119. Endo F, however, converted gp119 to a 69-kDa band and gp163 to a 122-kDa band. Intensity differences among preparations of the same protein (e.g., with or without enzyme) are due, at least in part, to the fact that each band shown here was derived from a separate starting band in the original preparative gel.

To confirm that these mobility shifts were due to the removal of appropriate oligosaccharide chains, the lectin-

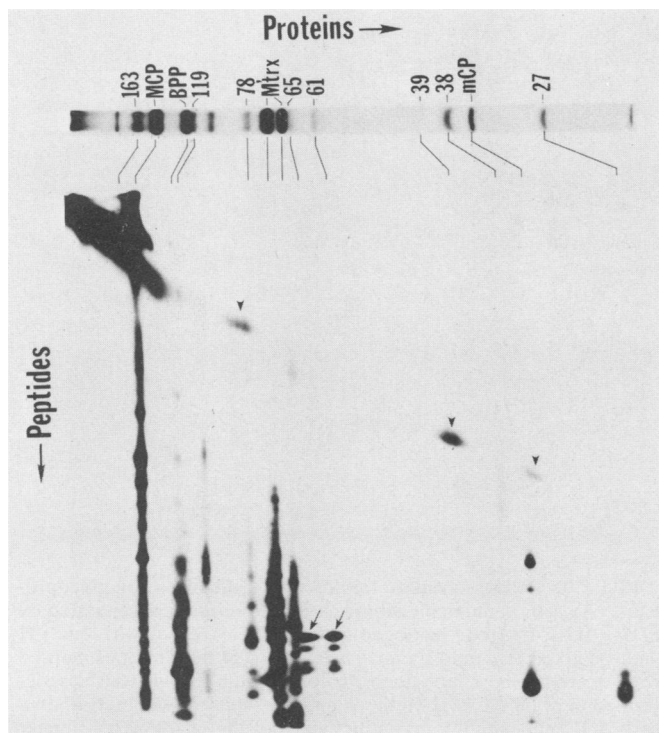


FIG. 7. Peptide comparisons of Colburn virion proteins. A polyacrylamide gel lane containing SDS-PAGE-separated, [<sup>35</sup>S]methionine-labeled Colburn virion proteins (similar to the one shown at the top of this figure) was applied to the top of a second gel (16% polyacrylamide cross-linked with *N,N'*-diallyltartardiamide) and subjected to partial proteolysis during the second-dimension SDS-PAGE. Shown here is a fluorogram prepared from the second gel. The two arrows (left to right) indicate gp65 and gp61 peptides, respectively; the three arrowheads (left to right) indicate the positions of gp87, gp39, and possibly gp36, respectively (Fig. 1, 2, and 8). Abbreviations: MCP, major capsid protein; BPP, basic phosphoprotein; M'trx, matrix proteins; mCP, minor capsid protein. Numbers indicate the molecular weight (in thousands) of the corresponding protein band.

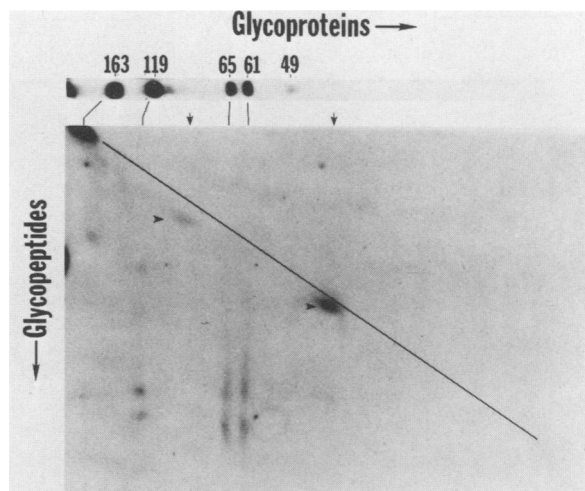


FIG. 8. ConA binding to Colburn virion glycoproteins after partial proteolysis. Colburn virion proteins were separated by SDS-PAGE. The sample-containing lane was sectioned out of the first gel and applied, perpendicular to the original direction of electrophoresis, to the top of a second gel (16% polyacrylamide, cross-linked with *N,N'*-diallyltartardiamide). After partial proteolysis during the second-dimension SDS-PAGE, the resulting polypeptides were electrotransferred onto nitrocellulose and incubated with <sup>125</sup>I-ConA. Shown here is a fluorographic image of the resulting nitrocellulose sheet. Numbers indicate molecular weights (in thousands) of the corresponding bands. The pattern at the top shows the same proteins visualized with <sup>125</sup>I-ConA after the first separation and before proteolysis. The diagonal line from the top left to the bottom right indicates the expected positions of proteins whose electrophoretic mobilities were unaffected by exposure to the protease. The two arrows at the top (left to right) indicate expected positions of gp87 and gp39, respectively (Fig. 1 and 7); the two arrowheads (left to right) near the diagonal line indicate the positions of corresponding ConA-binding proteins.

binding properties of endo-H- and endo-F-treated Colburn virion proteins were compared. Virus pellets prepared as described in Materials and Methods were resuspended in endo H or endo F buffer, disrupted by boiling for 1 min, combined with the respective enzyme (or buffer, in controls), and incubated overnight at 37°C. Treated and nontreated preparations were solubilized, subjected to SDS-PAGE, transferred onto nitrocellulose, and probed with ConA or WGA. Results, summarized in Fig. 10B, show that both endo H and endo F eliminated ConA binding. In contrast, endo H did not reduce WGA binding, and the effect of endo F on WGA binding was variable, ranging from a strong effect on some proteins to essentially no effect on gp163 (i.e., 163F). These findings are compatible with the specificities of these enzymes and lectins, as discussed below.

A similar experiment was done with iodinated proteins of HCMV (strain 751) virions. The following proteins were examined: (i) gp62, which has properties in common with Colburn gp65 (12) (Fig. 4, 5, and 6); (ii) the abundant, 69-kDa, lower matrix protein (12, 37); and (iii) proteins in the broad band containing gp145 and gp130 (Fig. 2 and 4). The gp145-gp130 band was cut in half in an attempt to enrich for gp145 (upper fragment) and gp130 (lower fragment). HCMV gp62 was decreased in apparent molecular weight to 60,000 by endo H and to 57,000 by endo F (Fig. 11). Neither enzyme altered the electrophoretic mobility of the 69-kDa matrix protein band (data not shown). The gel fragment enriched for gp145 yielded a 132-kDa band after endo H treatment and a

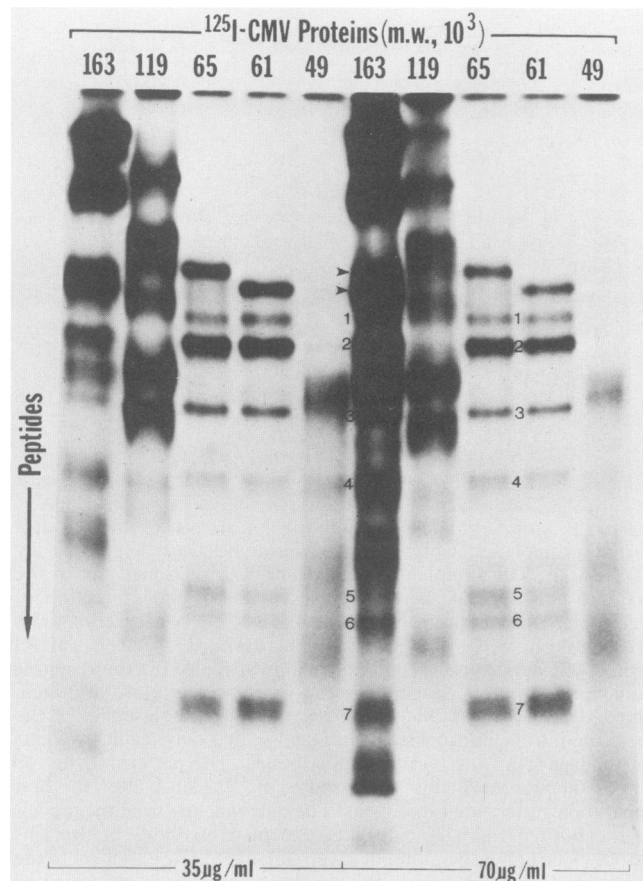


FIG. 9. Comparison of Colburn glycoprotein CNBr cleavage fragments. Iodinated Colburn virion proteins were separated by SDS-PAGE in a 7.5% polyacrylamide gel. Glycoprotein-containing bands were visualized by fluorography (33), excised, and exposed to CNBr (35 or 70  $\mu\text{g/ml}$ ) as described in Materials and Methods. Shown here is a fluorogram of the resulting peptide fragments after separation by SDS-PAGE in a 16% polyacrylamide gel cross-linked with *N,N'*-diallyltartardiamide. Arrowheads and numbers indicate comigrating peptides.

64-kDa band after treatment with endo F; the gel fragment enriched for gp130 yielded a 110-kDa band after endo H treatment and a broad, faster-migrating band after endo F treatment. The leading edge of this broad band migrated close to the position of the endo F cleavage product of gp62.

### DISCUSSION

Lectins and glycosidic enzymes have been used in conjunction with PAGE and electrotransfer to nitrocellulose to examine and compare the virion glycoproteins of SCMV and HCMV strains. The advantages of this approach are that (i) it does not require large amounts of starting material, which would be needed for direct biochemical determinations of carbohydrate structure and composition, and (ii) it is not affected by metabolic conversion and reutilization which can complicate biosynthetic radiolabeling studies.

Although this approach is not extensively documented, control experiments with CMV virion proteins gave results consistent with the described specificities of the lectins (WGA for sialic acid and ConA for high-mannose N-linked oligosaccharides), and the glycosidases (neuraminidase for sialic acid, endo H for high-mannose N-linked oligosac-

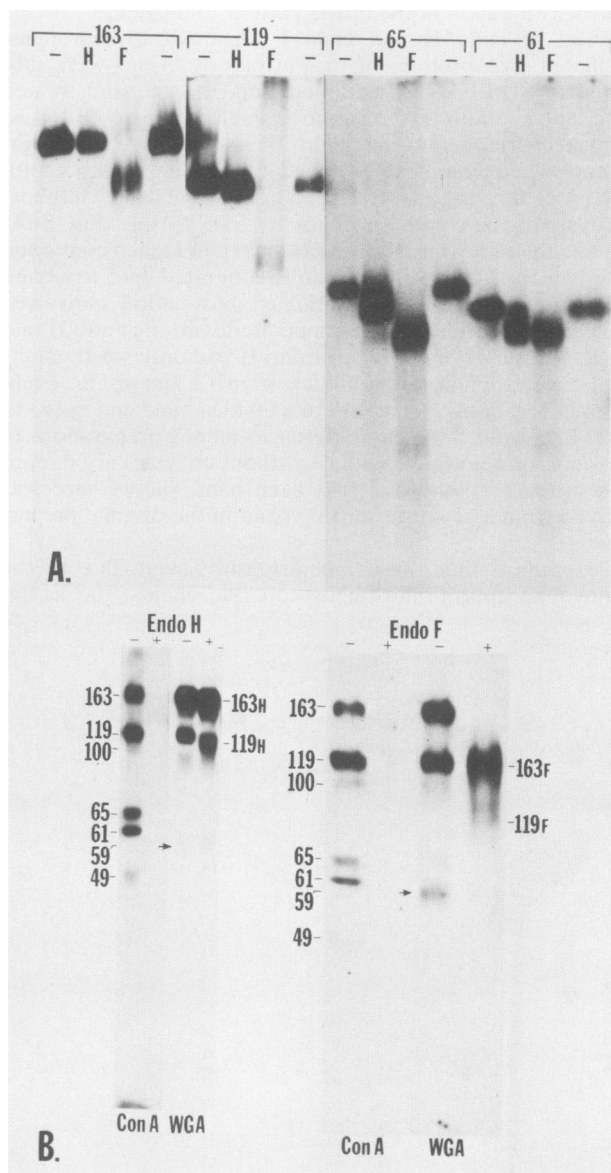


FIG. 10. Endoglycosidase treatment of Colburn virion glycoproteins. (A) Radioiodinated Colburn virion proteins were separated by SDS-PAGE, located, sectioned from the gel, treated with endo H (lanes marked H), endo F (lanes marked F), or buffer (lanes marked with a minus sign), and then analyzed after SDS-PAGE, as described in Materials and Methods and elsewhere in the text. Shown here is a composite fluorographic image of the resulting gel (longer exposure required for gp61 and gp65 lanes). (B) Nonlabeled Colburn virions were treated with endo H or endo F, as indicated, subjected to SDS-PAGE, electrotransferred onto nitrocellulose, and probed with  $^{125}\text{I}$ -ConA (lanes marked ConA) or  $^{125}\text{I}$ -WGA (lanes marked WGA), as described in Materials and Methods and elsewhere in the text. Shown here are fluorographic images of the resulting nitrocellulose replicas. Numbers indicate the molecular weight (in thousands) of the corresponding band. Positions of endoglycosidase-modified proteins are indicated by a number denoting their original molecular weight (in thousands) followed by a letter denoting the treatment used; these assignments are based on the results shown in panel A.



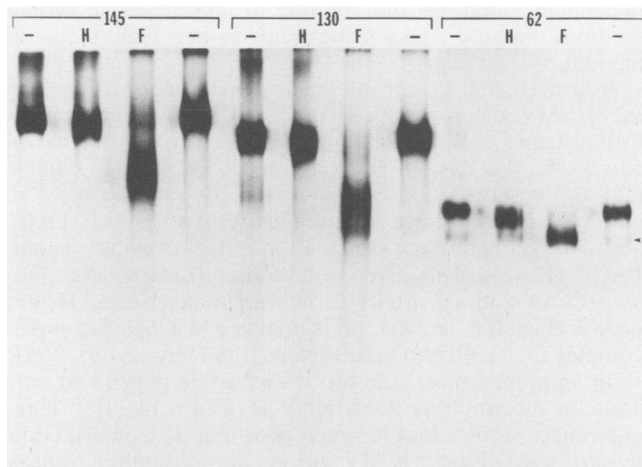


FIG. 11. Endoglycosidase treatment of HCMV virion glycoproteins. The procedure used was the same as that described in the legend to Fig. 10A. Details are presented in Materials and Methods and elsewhere in the text. Shown here is a fluorographic image of the resulting gel containing HCMV glycoproteins after treatment with endo H (lanes marked H) or endo F (lanes marked F) or after incubation without enzyme (lanes marked with a minus sign). Numbers indicate the molecular weight (in thousands) of the protein; the arrowhead at the right denotes the position of an apparent breakdown product of gp62.

charides, and endo F for high-mannose and complex N-linked oligosaccharides). While ConA is known to recognize certain other N-linked structures (21), the finding that endo H treatment abolished its binding indicates that this lectin recognized primarily the high-mannose type in our assays. The fact that endo F did not eliminate WGA binding, as might have been expected if all sialic acids were attached to N-linked carbohydrates, may be due to the following: (i) inaccessibility of some glycosidic bonds to the enzyme; (ii) the presence of endo-F-insensitive (e.g., O-linked), sialylated carbohydrates; or (iii) binding of WGA to N-acetylglucosamine residues exposed and left by endo F. Our results do not distinguish between the first two possibilities, but the third seems unlikely since the endo F preparation contained, and was used under conditions favoring, peptide:N-glycosidase (30). Because peptide:N-glycosidase cleaves at the asparagine bond, this preparation would be expected to remove the entire oligosaccharide.

The first series of experiments was done with strain Colburn, since its virion proteins, particularly the glycoproteins, are better resolved by SDS-PAGE than are their HCMV counterparts. Results showed that, of the eight bands recognized by lectins, seven (gp163, gp119, gp100, gp65, gp61, gp59, and gp39) correspond to bands provisionally designated as glycoproteins on the basis of their biosynthetic radiolabeling with [ $^3\text{H}$ ]glucosamine (12). Most of these (i.e., gp163, gp119, gp100, and gp65) reacted with both ConA and WGA, demonstrating that they contain both high-mannose N-linked and sialylated (e.g., complex N-linked or O-linked) carbohydrates. It is noteworthy that the degree of enhancement of WGA binding achieved with the cyanoborohydride-aniline pretreatment was inversely related to the size of these Colburn glycoproteins. The smallest, gp59, showed the greatest enhancement; the intermediate-sized band, gp119, showed moderate enhancement; and the largest, gp163, showed no enhancement with fresh aniline (Fig. 3). This may be due to an unusual arrangement

of sialic acid residues in the larger glycoproteins, such as clustering or a long chain structure, which itself enhances WGA binding. Glycoproteins gp49, gp59, and gp61 were distinguished by their reaction with only one lectin: gp59 with WGA, and gp49 and gp61 with ConA. These results, indicating the absence of high-mannose chains in gp59 and the absence of sialylated oligosaccharides in gp49 and gp61, are further discussed below. The observation that two glucosamine-labeled bands were not detected by lectin-binding assays (i.e., gp36 and gp27) may be explained by glucosamine incorporation into other molecules, such as glycolipids (e.g., gp36), or by the lack of assay sensitivity or lectin specificity (e.g., gp27).

This series of experiments also demonstrated that the acidic nature and charge heterogeneity of gp163, gp119, gp100, and gp65 are due to their sialic acid content and were dramatically altered after neuraminidase treatment (Fig. 5). Most notable of the neuraminidase-affected bands was gp163. Its net charge was changed from among the most acidic to among the most basic of the virion proteins, and its apparent size was increased by approximately 8%. Recent evidence from two-dimensional PAGE separations of iodinated material indicates the presence of a less acidic glycoprotein in the same size range as gp163 (unpublished observations). The relationship of these two proteins is presently unclear. Like gp163, gp59 had a slower electrophoretic mobility after neuraminidase treatment. These two glycoproteins are the most acidic Colburn virion proteins described, and their mobility shift is thought to result from an initially high ratio of sialic acid to total carbohydrate mass. Similar shifts have been observed with other highly sialylated glycoproteins (8).

The relationship between Colburn gp65 and gp61, also referred to as discrete glycoproteins 1 and 2 in recognition of their characteristic distribution after separation by two-dimensional PAGE (12), was further investigated. Peptide comparisons after enzymatic (V-8 protease) or chemical (CNBr) cleavage demonstrated that the two proteins are closely related. Evidence showing that gp61 contains high-mannose N-linked oligosaccharides (i.e., reacts with ConA; mobility altered by endo H) but neither complex (i.e., no further mobility change by endo F) nor sialylated (i.e., no WGA binding; no charge shift by neuraminidase) oligosaccharides indicates that gp61 is an incompletely processed form of gp65. The 5-kDa shift in electrophoretic mobility and the two partially deglycosylated bands observed after treatment of gp65 and gp61 with endo H (Fig. 10) suggest that both proteins contain two to three high-mannose N-linked carbohydrates. These appear to be either the same or located in the same region of both glycoproteins (Fig. 8). On the basis of its WGA reactivity, neuraminidase sensitivity, and further reduction (i.e., 4 kDa) in apparent size after treatment with endo F (Fig. 3, 5, and 10), gp65 is suggested to contain, in addition, one or two sialylated, complex N-linked oligosaccharides. The distribution of its charge isomers (Fig. 5) indicates that most gp65 molecules contain from one to three sialic acid residues and that only a small percentage remain nonsialylated (rightmost pair) or contain four sialic acids (leftmost spot). Recent studies have established that gp61 and gp65 are recovered as disulfide-linked heterodimers with gp119 in the virion envelope (D. M. Benko and W. Gibson, 10th Herpesvirus Workshop; manuscript in preparation). The similarity in electrophoretic mobility between the gp65-gp119 heterodimer and gp163 (data not shown) and the apparent peptide similarities between gp65 and gp163 (Fig. 9) are compatible with the possibility that gp163 may be

cleaved to yield gp119 and gp65, perhaps analogous to the precursor-disulfide-linked-product relationship that has been reported for the 120- to 125-kDa pseudorabies virion glycoprotein (16, 24).

When the virion proteins of additional SCMV strains were examined for comparison, it was found that they have counterparts to all of the Colburn glycoproteins. However, some variation occurred among these counterparts in their apparent size and relative reactivity with lectins. Notably, the electrophoretic mobility of Colburn gp119 was slower than its counterpart in other strains (Fig. 4; SCMV, panel WGA), whereas the electrophoretic mobilities of the strain SA6 gp65-gp61 counterparts were faster than those of the other strains (Fig. 4, SCMV, ConA panel). The observation that the SA6 and CSG gp65 counterparts, as well as the CSG gp163 and gp119 counterparts, reacted more strongly with ConA and were more diffuse than those of Colburn indicates that there is variation among these strains in the extent to which their high-mannose N-linked oligosaccharides are processed. However, since other nonglycosylated proteins differ in size among these SCMV strains (12) and because the relatively faster electrophoretic mobilities of the SA6 gp65 and gp61 bands were still observed after endo F treatment (data not shown), it is likely that at least some of these mobility differences are due to actual size differences in the proteins themselves.

The virion glycoproteins of HCMV strains were also examined. The patterns obtained from the lectin-binding assays (WGA in particular) were diffuse and reminiscent of those previously obtained from SDS-PAGE analyses of [<sup>3</sup>H]glucosamine-labeled virions (Fig. 4) (3, 12, 14, 32). Six glycoprotein bands were distinguished, including two previously identified by using an analogous gel overlay procedure (27). Two bands, gp145 and gp130, moved as a broad doublet zone just beneath the 150- to 155-kDa major capsid protein and the basic phosphoprotein (12, 14). The relative positions of these two bands appear to correspond to the 132- to 115-kDa (Fig. 10B in reference 32), 130- to 94-kDa (Fig. 1d in reference 27), 130- and 95-kDa (Fig. 5a in reference 7), 116-kDa (Fig. 2 in reference 3), and 92-kDa pair (Fig. 2-1 in reference 31) bands described by others. These two glycoproteins were also distinguished by their reactivities with lectins: gp145 reacted much more strongly with WGA than with ConA, whereas gp130 showed the opposite reactivities (Fig. 4). More recent comparisons of these two glycoproteins, based on two-dimensional separations (charge-size; nonreducing-reducing) of iodinated virion proteins, have shown the following. (i) gp145 is acidic, whereas gp130 is comparatively neutral. (ii) gp145 does not have intermolecular disulfide cross-links, whereas gp130 is recovered from the virion as a disulfide-linked heterodimer with gp62 (D. M. Benko and W. Gibson, *Abstr. Annu. Meet. Am. Soc. Microbiol.* 1986, S6, p. 310; manuscript in preparation).

Two other virion glycoproteins detected by WGA, gp70 (not previously described) and gp57 (counterpart to Colburn gp59), were poorly resolved by SDS-PAGE alone (Fig. 4). After two-dimensional separations (charge-size) of iodinated virion proteins, however, these species were clearly distinguished and, along with gp145, represent the most acidic virion proteins detected (Benko and Gibson, 10th Herpesvirus Workshop; in preparation). gp145, gp70, and gp57 were less readily detected by WGA in strains AD169 and Towne than in strains Davis and 751 (Fig. 4). Because estimates based on ConA binding (Fig. 4) and silver staining indicated that these HCMV virion preparations contained roughly equivalent amounts of other proteins, these apparent strain-

dependent variations may be due to differences in protein modification (e.g., less sialic acid) rather than to actual amount.

A fifth HCMV glycoprotein, designated here as gp62, is the HCMV discrete glycoprotein and the counterpart of Colburn gp65 (12). Size estimates of this protein range from 52 (7), 55 (31), 58 (27), 58.5 (29), and 62 kDa (Fig. 2 and 4) (12) to 64 kDa (32), but it is readily identified by its comparatively discrete banding pattern after SDS-PAGE and its characteristic distribution after two-dimensional PAGE (12, 14). This glycoprotein also (i) shows reactivity with ConA and sensitivity to neuraminidase, endo H, and endo F (Fig. 4, 6, and 11), (ii) is involved in a disulfide-linked complex (3, 31; Benko and Gibson, 10th Herpesvirus Workshop; in preparation), and (iii) shows strain-dependent variation in electrophoretic mobility (Fig. 4) (14, 27). Three differences were found between gp62 and its Colburn counterpart, gp65. First, HCMV virions do not contain a direct counterpart to Colburn gp61 (i.e., a band of lower molecular weight was not detected by ConA or polyclonal antibodies to gp62 (Fig. 2 and 4; unpublished results), which was shown here to be an incompletely modified form of gp65. Second, estimates based on electrophoretic mobility shifts produced by endo H and endo F (Fig. 11) indicate that gp62 contains fewer N-linked carbohydrates than gp65, perhaps only one high-mannose and one complex type. Third, the charge heterogeneity of gp62 was less affected by neuraminidase than that of Colburn gp65 (Fig. 5 and 6). The failure of neuraminidase to convert the acidic gp62 charge isomers to more basic forms may be due to the presence of neuraminidase-resistant (e.g., N-substituted or O-substituted) sialic acids (36). Alternatively, other features of the protein or its environment may be responsible for the charge heterogeneity and neuraminidase resistance of gp62. Similarities in size and charge between this CMV glycoprotein species and herpes simplex virus gD have been noted before (12, 14). A sixth band, gp163 (Fig. 2 and 4, HCMV, panel ConA), probably corresponding to the 160-kDa glycoprotein described by Britt (3), was not effectively visualized in all experiments. Nonreducing and two-dimensional (nonreducing-reducing) separations indicate that the 163-kDa protein (i) is disulfide-linked to gp130 and gp62, as reported by others (3), or (ii) is disulfide-linked to itself and comigrates with a 300-kDa gp130-gp62 tetramer (Benko and Gibson, 10th Herpesvirus Workshop; *Abstr. Annu. Meet. Am. Soc. Microbiol.* 1986; manuscript in preparation).

In summary, these studies have provided additional information about the virion glycoproteins of both SCMV and HCMV strains. Most, if not all, of those identified contained N-linked oligosaccharides. Among them, both high-mannose and complex types were distinguished. The presence of sialic acid was also demonstrated, and the finding that endo F did not eliminate WGA binding to all of the glycoproteins (e.g., gp163) suggests that sialylated O-linked carbohydrates, as well as sialylated N-linked carbohydrates, are present. The additional findings that HCMV gp62 does not have an immature form in virions (i.e., a counterpart to Colburn gp61), that its charge heterogeneity was only partially reduced by neuraminidase, and that the WGA-binding pattern for HCMV strains was significantly more diffuse than the corresponding pattern for SCMV strains suggest that HCMV glycoproteins are more extensively processed than their Colburn counterparts. This possibility is of particular interest since it implies differences in the maturational events related to glycoprotein processing between HCMV and SCMV strains grown in the same cell type.

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