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Synthesis and Processing of the Envelope gp55-116 Complex of Human Cytomegalovirus

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The envelope of human cytomegalovirus has been reported to contain between three and eight glycoproteins. Major constituents of the envelope include two abundant glycoproteins with estimated molecular weights of 55,000 (gp55) and 116,000 (gp116). These two glycoproteins have been shown to exist as a disulfide-linked complex (gp55-116) within the envelope of mature virions. Utilizing a panel of monoclonal antibodies reactive with the gp55-116 complex, we characterized the synthesis and processing of these two virion proteins. Infected cells were shown to contain two glycosylated proteins of 160,000 and 150,000 daltons as well as the mature gp55 and gp116. Pulse-chase analysis indicated that gp150 was a precursor protein of gp160. The mature gp55 and gp116 were generated, in turn, by cleavage of gp160. Antigenic and structural analysis revealed that gp55 and gp116 shared little structural homology and no detectable antigenic cross-reactivity. The results of this study are discussed in relation to the synthesis of envelope proteins of other herpesviruses.

Cytomegalovirus (CMV) represents an important cause of morbidity and mortality in immunocompromised hosts (12). In addition, congenital CMV infections account for a significant number of developmentally disabled children every year in the United States (1). Although in previous studies a variety of specific immune responses to CMV in infected patients has been documented (2, 18, 23–25), it remains unclear which responses are critical for limiting infection in vivo. Furthermore, little is known about the immune response to specific CMV-encoded proteins. Results of recent studies have suggested that the envelope glycoproteins are immunogenic in humans and may be important targets for the immune response. Sera from patients convalescing from CMV infections can neutralize infectious virus in vitro and have been shown to contain antibodies that are reactive with these glycoproteins (3, 20). This finding suggests that antibodies against these glycoproteins may also be active in the in vivo clearance of CMV. Additional evidence has also suggested that these same envelope glycoproteins are expressed on the surface of infected cells (20). Recognition of these cell surface viral antigens by immunological effector functions such as cytotoxic T lymphocytes or antibody-dependent cellular cytotoxicity or both could result in the elimination of virus-infected cells. Thus, a further understanding of the role of the immune response in the control of CMV infections will necessarily require a thorough characterization of the CMV glycoproteins.

In earlier studies the existence of between three and eight electrophoretically distinct glycoproteins within the envelope of CMV has been demonstrated (3, 7, 8, 10, 14, 17, 26, 27). Because many of these investigations were done without the aid of immunological techniques, the actual number of antigenically and structurally unique proteins in the CMV envelope is unclear. In fact, more recent studies have suggested that the envelope of CMV may contain a limited number of polymorphic glycoproteins (21). In a previous study, we utilized CMV-specific monoclonal antibodies to define a disulfide-linked envelope protein complex consisting of three glycoproteins with estimated molecular weights of 160,000 (gp160), 116,000 (gp116), and 55,000 (gp55) (3). These proteins were initially detected in virus from extracellular supernatants prepared by centrifugation of supernatant fluid from infected cells. Subsequent studies, in which an additional purification step of sedimentation of this virus-enriched material through sorbitol density gradients was included, revealed that infectious virions contained only gp55 and gp116 (4; W. J. Britt, manuscript in preparation). In this report we describe the results of our studies on the synthesis of this envelope protein complex and its various intracellular forms. Our findings indicated that the mature gp55-116 complex was generated by cleavage of a glycosylated 160-kilodalton (kDa) precursor protein. This gp160 precursor protein was synthesized from a glycosylated 150-kDa precursor protein. Antigenic and structural analysis indicated that gp55 and gp116 were dissimilar, suggesting potentially different structural roles for these envelope proteins.

MATERIALS AND METHODS

Cells and virus. The propagation of human foreskin fibroblasts has been described previously (3). CMV strain AD169 was originally obtained from Barry Hanshaw, University of Massachusetts, Worcester, Mass. The purification of this virus from extracellular supernatants has been described in an earlier report (3).

Monoclonal antibody productions. The production and characterization of the antibodies used in this study have been described previously (3). All antibodies were classified as CMV specific in that they were nonreactive in an immunofluorescence assay (3) with either herpes simplex or varicella-zoster virus-infected human fibroblasts as well as noninfected fibroblasts. All hybrid cell lines were cloned through two successive cycles of limiting dilution cloning before use. Monoclonal antibody 7-17 has been shown to react with the gp55-116 complex in gradient-purified virions as well as gp160, gp116, and gp55 in extracellular, partially purified virus (3). This antibody neutralizes infectious virus in vitro (3). Antibodies 27-11, 27-83, 27-160, 27-180, and 27-283 were produced in an identical fashion as antibody 7-17. Of this group, only antibody 27-283 neutralized infec-

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tious virus. Antibody 48, a murine monoclonal antibody that is reactive with Friend murine leukemia virus gp70 (5), was used as a control for nonspecific reactivity.

Radiolabeling of infected-cell proteins. Confluent monolayers of human fibroblasts in 60-mm-diameter tissue culture dishes were infected with AD169 at a multiplicity of infection of 1. Four days postinfection the cells showed evidence of early cytopathic effect and were labeled overnight with [^{35}S]methionine and [^{3}H]glucosamine as described previously (3). The cells were harvested 24 h later by first washing the monolayers three times with Dulbecco modified phosphate-buffered saline (DPBS; pH 7.3) followed by the addition of 1.5 ml of extraction buffer which was composed of 0.1% sodium dodecyl sulfate (SDS), 1.0% Nonidet P-40, and 1.0% deoxycholate in tris-buffered saline (TBS; 0.05 M Tris, 0.15 M NaCl; pH 7.3). The detergent-containing solution was allowed to incubate on the monolayers for 15 min at 4°C, and the detergent-solubilized cell lysates were then stored at −70°C. In all experiments 0.1 mM phenylmethylsulfonyl fluoride (Sigma Chemical Co., St. Louis, Mo.) was added to the extraction buffer as well as to the wash buffer used in immune precipitation procedures.

Immune precipitation and SDS-polyacrylamide gel electrophoresis. The radiolabeled lysates were precleared by incubation with normal mouse serum and Formalin-fixed *Staphylococcus aureus* (Bethesda Research Laboratories, Gaithersburg, Md.) at 4°C for 2 h. The lysates were then clarified by centrifugation at 75,000 × *g* for 45 min. The precleared lysates were incubated overnight at 4°C with tissue culture supernatant containing monoclonal antibodies, and the immune complexes were collected by adding *S. aureus*. After four washes with cold extraction buffer, the pellets were eluted in 50 mM TBS containing 2% SDS and 5% 2-mercaptoethanol (2-ME), or an identical buffer except without 2-ME, and heated to 100°C for 3 min. The samples were applied to 10 or 7.5% acrylamide gels as described previously (3). Molecular weights were estimated by comparison with the migration of standard molecular weight markers (Sigma).

Pulse-chase analysis of infected-cell proteins. Briefly, CMV-infected monolayers in 60-mm-diameter tissue culture dishes were deprived of methionine by incubation in DPBS for 30 min. The monolayers were then pulse labeled for 10 min with 50 μCi of [^{35}S]methionine per ml. The radioactive medium was removed, and normal maintenance medium was added. The cells were then incubated for various times, and the chase period was terminated by first washing the dish in DPBS and then rapidly freezing the entire dish at −70°C. The monolayers were then thawed, and the infected cell proteins were extracted as described above.

Western immunoblotting. The details of Western immunoblotting have been described previously (5). Briefly, infected monolayers of cells were removed from tissue culture dishes with a rubber policeman. The cells were pelleted, SDS-polyacrylamide gel electrophoresis (PAGE) elution buffer was added, and the mixture was heated to 100°C for 3 min. Virus obtained by centrifugation through sorbitol density gradients was solubilized in an identical manner. The virus or cell lysate was then subjected to SDS-PAGE. The separated proteins were then transferred to nitrocellulose (NC) membranes with a trans blot apparatus (Bio-Rad Laboratories, Richmond, Calif.). The transfer buffer and length of transfer were carried out as described previously (5). The membrane was blocked in 2% bovine serum albumin–TBS for 2 h at 37°C, cut into strips, and then reacted with tissue culture supernatant containing monoclonal antibodies overnight at 4°C. The strips were washed in 0.05% Tween 20 (Sigma)–TBS several times, and approximately 10^6 cpm of ^{125}I-rabbit anti-mouse immunoglobulin (Pel-Freeze, Ft. Smith, Ark.) per ml was added. After a 45-min incubation, the strips were again washed, dried, and exposed to film.

Proteolytic peptide mapping. The proteolytic peptide mapping procedure was an adaptation of that of Cleveland et al. (6). Briefly, [^{125}I]-iodine labeled virus was prepared by reacting approximately 20 μg of gradient-purified virions in DPBS with 1.0 mCi of [^{125}I]iodine in the presence of 20 μg of chloramine T (Sigma). Following a 2-minute incubation at 25°C, 40 μg of sodium metabisulfite in DPBS was added. The mixture was then dialyzed extensively overnight at 4°C in DPBS. Intact labeled virions were lysed by making the final mixture 1.0% SDS by the addition of 20% SDS. The solubilized proteins were precleared as described previously and reacted with monoclonal antibody 7-17. Following separation by SDS-PAGE, gp55 and gp116 were visualized by autoradiography, and the appropriate bands were cut from the gel. The gel slices were washed three times in 100% methanol, dried, and then placed in the wells of a second 15% acrylamide gel. Various concentrations of staphylococcal V8 protease (SV8; Miles Laboratories, Naperville, Ill.), or trypsin (Sigma) in TBS were added. The gel slices and enzymes were electrophoresed until the residual tracking dye from the gel slices reached the interface of the stacking and resolving gel. The power was then turned off, and digestion was allowed to progress for 45 min. Electrophoresis was then resumed. Proteolytic peptide mapping of intracellular forms of the gp55-116 complex was carried out in a similar fashion, except that the infected cell proteins were labeled with 100 μCi of [^{35}S]methionine per ml as described above. Following solubilization in extraction buffer, the infected cell proteins were precipitated with antibody 7-17 and separated by SDS-PAGE. Molecular weights were estimated by the position of the radiolabeled peptides as compared with the migration of known standards to which cytochrome *c* (Sigma) was added.

RESULTS

The gp55-116 envelope protein complex of CMV is synthesized from a 150-kDa precursor protein. The intracellular forms of the CMV gp55-116 envelope complex were studied by using a group of CMV-specific murine monoclonal antibodies reactive with this protein complex. Detergent-solubilized proteins from [^{35}S]methionine pulse-labeled, CMV-infected human fibroblasts were precipitated with antibody 7-17 and analyzed by SDS-PAGE under reducing conditions. Antibody 7-17 precipitated three broadly migrating proteins with estimated molecular masses of 55, 116, and 160 kDa as well as a more discretely migrating protein of 150 kDa (Fig. 1, +2-ME). The 160-kDa protein migrated as a diffuse band between 150 and 160 kDa and was not readily resolved from the discrete 150-kDa protein in this gel system (Fig. 1). Human immune sera WB precipitated a larger number of proteins, some of which comigrated with proteins precipitated by antibody 7-17 (Fig. 1). Nonimmune control sera KF, as well as antibody 7-17 and sera WB, nonspecifically precipitated proteins of 68, 49, and 30 kDa (Fig. 1). Antibody 7-17 also precipitated three proteins of 55, 116, 150, and 160 kDa from [^{3}H]glucosamine-labeled CMV-infected cells (Fig. 1, +2-ME), although the gp55 incorporated much less [^{3}H]glucosamine than the 116-, 150-, or 160-kDa proteins and was not easily seen in this reproduction of the original fluorogram (Fig. 1). It should be noted

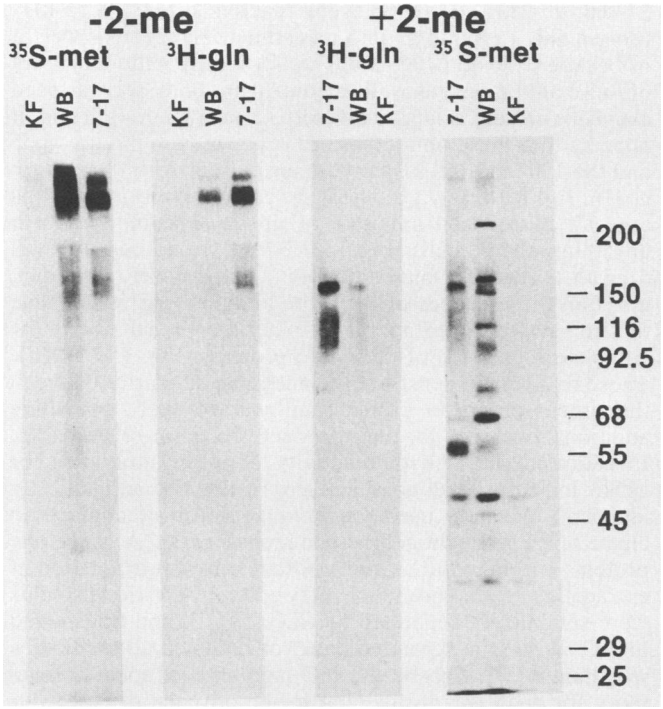

FIG. 1. SDS-PAGE analysis of immune precipitated proteins from [^{35}S]methionine- (^{35}S-met) and [^{3}H]glucosamine- (^{3}H-gln) radiolabeled, CMV-infected human fibroblasts. Monoclonal antibody 7-17, convalescent immune human sera WB, and control nonimmune human sera KF were used as the source of precipitating antibodies. Precipitated proteins were eluted in the presence (+) or absence (−) of 2-ME and analyzed in 7.5% acrylamide gels. Molecular weights (in kilodaltons) are shown on the right.

that the 116-kDa protein exhibited very broad migration ranging from 105 to 116 kDa but was arbitrarily assigned a molecular weight of 116 kDa. These findings document the presence of previously described 160-, 116-, and 55-kDa glycoproteins (3) within infected cells and identify a 150-kDa glycosylated protein not found in partially purified virion preparations. When identically prepared immune precipitates from either [^{3}H]glucosamine- or [^{35}S]methionine-labeled infected cell proteins were eluted in SDS containing buffer in the absence of the reducing agent 2-ME, a number of larger proteins, ranging in size from 160 to 180 kDa and >200 kDa were observed (Fig. 1, −2-ME). In addition, gp55 and gp116 were no longer detectable in the absence of 2-ME, indicating that these envelope glycoproteins exhibited extensive disulfide bonding as previously reported (3).

To delineate the precursor-product relationship of these envelope glycoproteins, we used pulse-chase analysis of infected-cell proteins. After a 10-min pulse with [^{35}S]methionine in methionine-deficient medium, infected cells were incubated in medium containing normal concentrations of nonradiolabeled methionine for various times. Antibody 7-17 and a control antibody, 48, were used to precipitate detergent-solubilized proteins from these infected cells. Antibody 7-17 precipitated a 150-kDa protein which incorporated radiolabeled methionine during the initial pulse labeling period (Fig. 2). The amount of the 150-kDa protein increased during the chase period, and 40 min after the initial pulse, a more diffusely migrating 160-kDa protein was detectable. In the following chase period (80 min), the diffusely migrating 160-kDa protein became more apparent, and proteins of 116 and 55 kDa were demonstrable (Fig. 2). In chase periods after 80 min, the 160-, 116-, and 55-kDa proteins accumulated, whereas the amount of the 150-kDa protein decreased slightly (Fig. 2). This processing pathway appeared to occur relatively slowly, as evidenced by the detection of gp55 and gp116 some 80 min after the initial pulse and by the continued presence of the 150-kDa precursor 24 h after the pulse (Fig. 2). These results suggest that the gp55-116 complex of CMV is synthesized as a 150-kDa precursor which is then presumably further glycosylated to a 160-kDa form. Shortly thereafter, or possibly concurrently, gp160 is cleaved into mature gp55 and gp116 envelope proteins.

Although the results of the pulse-chase experiment strongly suggest that the mature gp55-116 complex is a cleavage product of the gp160 precursor protein, we further investigated the structural relationship of the precursor and mature proteins by proteolytic peptide mapping. Infected-cell proteins were radiolabeled with [^{35}S]methionine, immune precipitated with monoclonal antibody 7-17, and separated by SDS-PAGE. Following identification of gp160, gp150, gp116, and gp55 by autoradiography, the gel slices containing these radiolabeled proteins were excised from the gel and digested with SV8 as described previously by Cleveland et al. (6). The resulting peptide fragments were then separated by SDS-PAGE. When the two forms of the precursor protein, gp160 and gp150, were digested with various amounts of SV8, the resulting peptide profiles appeared indistinguishable, indicating that these two proteins are very similar structurally (Fig. 3). Comparison of the

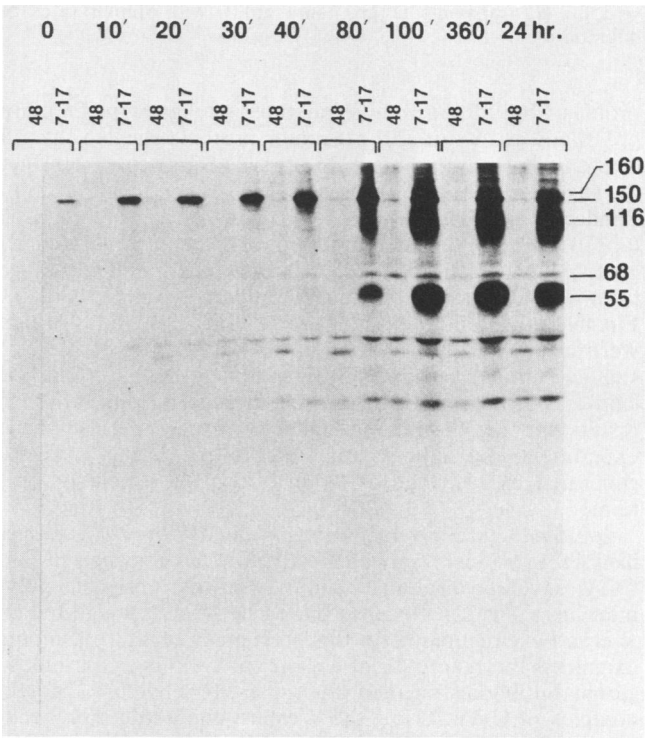

FIG. 2. Pulse-chase analysis of CMV-infected human fibroblasts. CMV-infected cells were radiolabeled with [^{35}S]methionine as described the text and precipitated with monoclonal antibody 7-17 or control antibody 48, and the immune precipitates were analyzed by SDS-PAGE. The chase periods are listed above the gels, and the molecular weights (in kilodaltons) of the precipitated proteins are shown on the right.

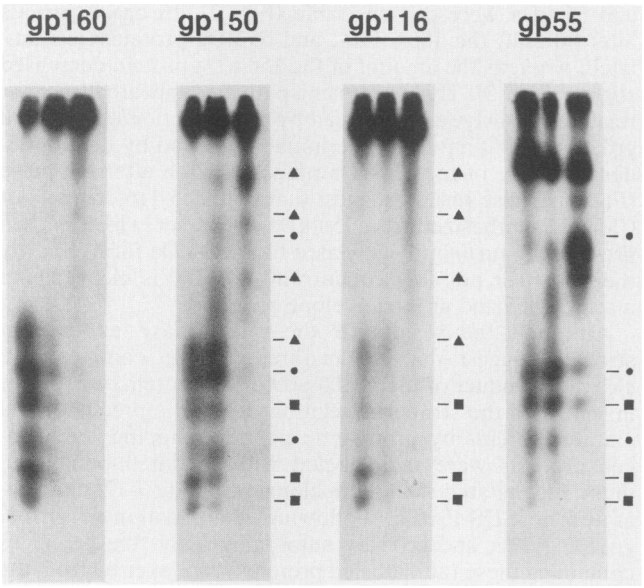

FIG. 3. Proteolytic peptide mapping of intracellular forms of the gp55-116 complex. Infected-cell proteins were radiolabeled with [^{35}S]methionine, solubilized, and immune precipitated with monoclonal antibody 7-17. Following separation by SDS-PAGE, the gel slices containing gp160, gp150, gp116, and gp55 were excised and digested with 1, 0.1, and 0.001 μg (in each of the lanes from left to right, respectively) of SV8 protease in the wells of a second, 15% acrylamide gel. The peptide fragments were then electrophoretically separated. Symbols: ▲, peptide fragments of gp116 which were also found in gp160 (gp150); ●, fragments found in both gp55 and gp160 (gp150); ■, fragments in gp55 and gp116 with similar rates of migration.

profile of peptide fragments generated by digestion of mature gp116 and gp160 (gp150) precursor protein revealed that all of the peptides found in gp116 are also found in gp150 (Fig. 3), suggesting that gp116 is generated from gp160 (gp150). Similarly, the peptide profile of precursor protein gp160 (gp150) also contained the peptide fragments generated by proteolytic digestion of gp55, suggesting that this mature protein is also derived from this precursor protein (Fig. 3). Finally, in addition to the several unique peptides which were not shared between gp116 and gp55 (Fig. 3), several smaller peptides generated by digestion of these proteins had similar, but not identical, rates of migration (Fig. 3). These results support the findings gathered from the pulse-chase experiment and indicate that mature gp116 and gp55 are generated by cleavage of the larger gp160 precursor protein.

gp116 and gp55 envelope proteins of CMV are antigenically distinct. Previously, other investigators have suggested that CMV envelope proteins exhibit extensive antigenic polymorphism (20, 21). Because our earlier results suggested an alternative explanation for this presumed polymorphism, we examined the reactivity of a panel of CMV-specific monoclonal antibodies against the gp55-116 envelope protein complex of CMV. Using the Western immunoblotting technique, we studied the reactivity of these antibodies against denatured and reduced proteins, thereby eliminating any polymorphism which may have arisen as the result of complex formation between individual proteins. After separation by SDS-PAGE and transfer to NC membranes, infected-cell proteins were reacted with monoclonal antibodies shown to be specific for the gp116-gp55 complex. Antibodies 27-160, 27-283, and 27-83 were reactive against a 55-kDa protein and a protein(s) with an estimated molecular weight of between 150 and 160 kDa (Fig. 4). Nonspecific reactivity of these antibodies, as well as control antibody 48, was seen against a protein of approximately 68 kDa (Fig. 4). Thus, it appears that these antibodies are reactive with mature gp55 and the 150- and 160-kDa precursor but not with the mature gp116. In Fig. 4 only gp150 can be readily seen, possibly as a result of reduced amounts of gp160 as compared with gp150 in infected cells (Fig. 1, +2-ME). In addition, it was difficult to identify small amounts of the diffusely migrating gp160 in the presence of the more abundant gp150 because the gel system used in these experiments did not allow complete separation of these two proteins (Fig. 1, +2-ME). These findings demonstrate the antigenic similarity between the precursor gp150 protein and mature gp55, providing additional evidence for their product-precursor relationship. In addition, the lack of reactivity of these antibodies for gp116 indicates that gp55 and gp116 are not antigenically identical. We next used the immunoblotting technique to characterize the antigenic relatedness of these envelope proteins within mature virions obtained by centrifugation of extracellular virus through density gradients. Virion proteins were solubilized, separated by SDS-PAGE, and transferred to NC. When an expanded panel of monoclonal antibodies was then used to probe the NC membranes, specific reactivity for gp55 was again seen in the absence of reactivity against gp116 (Fig. 4). In agreement with previous results (4), the precursor forms of the complex, gp150 and gp160, were not detected in this preparation of mature virions (Fig. 4). Nonspecific reactivity for a 68-kDa protein was also seen with control antibody 48 as well as the antibodies reactive with gp55 (Fig. 4). These results demonstrate that a number of monoclonal antibodies could distinguish antigenic heterogeneity between the gp116 and gp55 envelope proteins of CMV, suggesting that components of the gp55-116 envelope complex are antigenically distinct, even though they were derived from a common precursor.

The gp116 and gp55 envelope proteins exhibit little structural homology. The lack of shared antigenicity between gp116 and gp55 suggests that these proteins are also structurally different. In addition, although proteolytic peptide mapping revealed some apparently common peptide fragments in gp116 and gp55, the finding of several unique peptide fragments in each protein suggests that major structural heterogeneity exists between these proteins. To further explore this possibility we again used proteolytic peptide mapping as a method to compare these two envelope proteins. However, in the following experiments we used extracellular, gradient-purified virions as a source of gp116 and gp55. This source of envelope proteins offered important advantages over the use of infected-cell proteins. First, because gp150 was not present in the mature virion, there was little chance of contaminating gp116 with small amounts of comigrating gp150 (Fig. 2). In addition, it allowed us to study the relationship between the mature forms of gp116 and gp55 and the not incompletely processed forms of these proteins or precursor proteins or both which may have comigrated with either gp116 or gp55. Extracellular CMV virions were purified by centrifugation through a density gradient and radiolabeled with [^{125}I]iodine. Following disruption in detergent-containing buffer, the gp116 and gp55 were immune precipitated with antibody 7-17 and separated by SDS-PAGE. The radiolabeled proteins were then subjected to proteolytic peptide mapping as described above. Digestion of gp55 with increasing amounts of SV8 resulted in

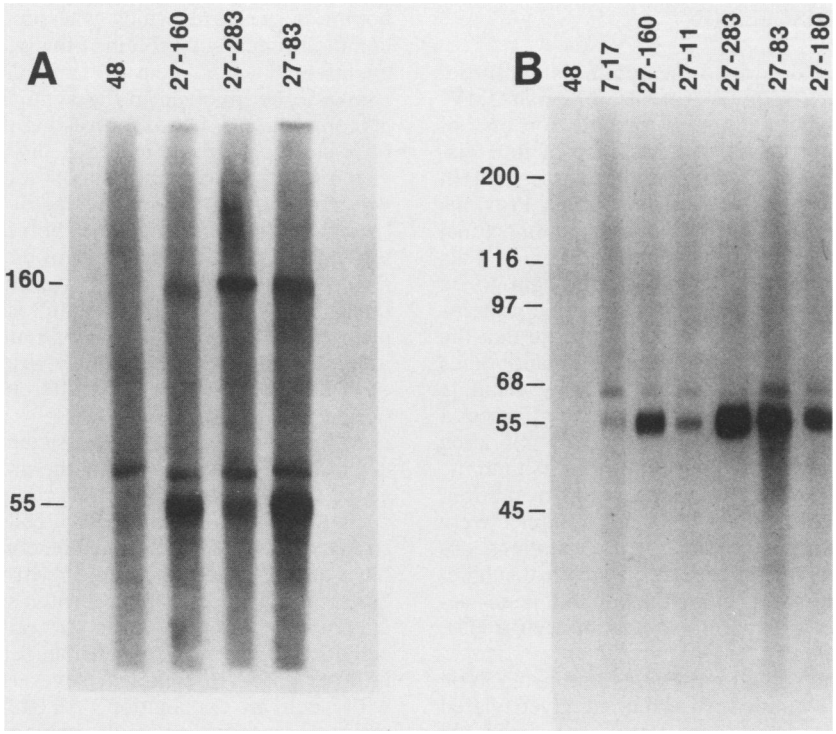

FIG. 4. Western blot analysis of CMV-infected cell proteins and sorbitol gradient-purified extracellular virions. CMV-infected cells and purified virions were disrupted as described in the text, subjected to SDS-PAGE, and transferred to NC membranes. The separated proteins were then reacted with control antibody 48 and CMV-specific monoclonal antibodies 7-17, 27-11, 27-83, 27-160, 27-180, and 27-283. Reactivity was detected by ^{125}I-labeled rabbit anti-mouse immunoglobulin (Fab_2). Reactivity against infected-cell proteins (A) and virions (B) is shown. Molecular weights (in kilodaltons) are shown on the left margin of each panel.

the appearance of a 30-kDa fragment and two fragments with estimated molecular masses of 10 and 15 kDa (Fig. 5). In contrast to these findings, only a diffusely migrating fragment with an estimated molecular mass of 40 kDa and three more rapidly migrating fragments were observed when gp116 was digested with SV8 (Fig. 5). Digestion of gp55 and gp116 with trypsin resulted in the generation of an increased number of fragments as compared with treatment with SV8 (Fig. 5). None of the fragments generated by SV8 or trypsin treatment of gp55 and gp116 comigrated in the 15% gels used for these analyses (Fig. 5). In addition, the patterns of sequential proteolytic digestion were clearly different for gp116 and gp55 (Fig. 5). These results indicate that gp55 and gp116 share little, if any, sequence homology. Thus, it appears that gp55 and gp116 not only differ in their antigenicity but also in their structure.

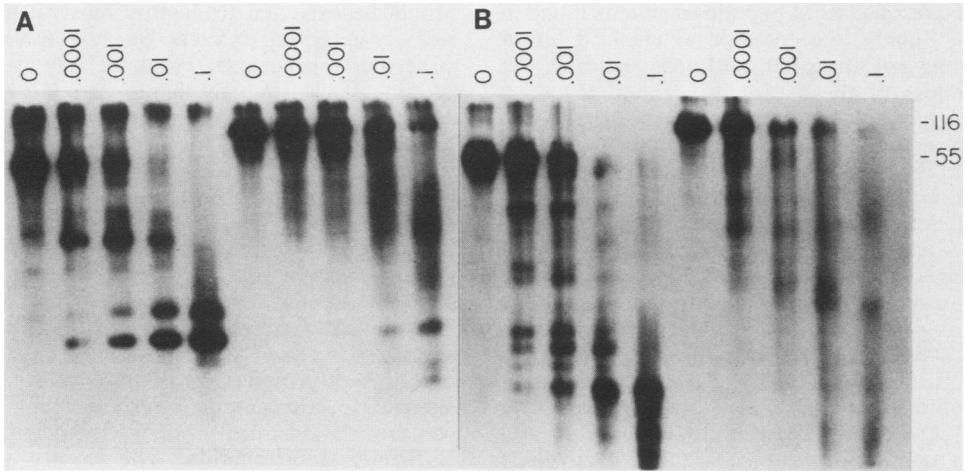

FIG. 5. Proteolytic peptide mapping of gp55 and gp116 of extracellular virus. Radiolabeled virions were disrupted and immune precipitated with antibody 7-17, and the precipitated gp55 and gp116 were separated by SDS-PAGE. The gel slices containing gp55 and gp116 were then placed in a second gel, and various concentrations (in micrograms; indicated above the gels) of either SV8 (A) or trypsin (B) were added. Following digestion, the radiolabeled peptides were electrophoretically separated in 15% acrylamide gels.

DISCUSSION

In this report we have described the synthesis and processing of two major envelope glycoproteins of human CMV. Analysis with monoclonal antibodies specific for one of these envelope proteins revealed the presence of four distinct glycoproteins with estimated molecular masses of 160, 150, 116, and 55 kDa within CMV-infected cells. Previous studies have also shown that CMV-specific monoclonal antibodies recognize multiple proteins within infected cells (20, 21). This apparent polymorphism was thought to be secondary to multiple forms of individual envelope proteins within infected cells. However, our results indicate that the multiple reactivities exhibited by the monoclonal antibodies used in this study can be explained, in part, by disulfide bonding between these envelope proteins. In the absence of reducing agents, such as 2-ME, only more slowly migrating species with equivalent or higher molecular weights than the most slowly migrating precursor were observed. In addition, none of the mature forms of the gp55-116 complex were detectable in the absence of 2-ME. These findings are consistent with those of recent reports in which disulfide-linked envelope glycoprotein complexes have been described in bovine herpes virus (16), pseudorabies virus (11), and varicella-zoster virus (9).

Using a group of monoclonal antibodies and proteolytic peptide mapping, we identified two related glycosylated precursor proteins in the synthetic pathway of the gp55-116 complex. Several findings indicate that gp150 and gp160 are precursors of the mature gp55-116 complex. First, gp150 and gp160 appeared to be precursors found in infected cells and not in virions because neither of these forms was detected in virions prepared by sedimentation of extracellular virions through sorbitol density gradients. This result was also in agreement with those of recent reports in which have been described a number of envelope glycoproteins of CMV, none of which had an estimated molecular mass of between 140 and 170 kDa (7, 17). Second, the sequential synthesis of gp150 and gp160 followed by the appearance of mature gp55 and gp116 indicates that there is a temporal relationship in the synthesis of the mature proteins from the gp160 precursor. Furthermore, proteolytic peptide mapping revealed structural homology between gp160 and gp150 and also indicated that the peptide fragments generated by digestion of gp116 and gp55 are a subset of peptide fragments found in gp160 and gp150. Finally, the common antigenic determinants shared among gp150, gp160, and gp55 indicates the structural relatedness of these proteins. Taken together, these results strongly suggest that the mature gp55 and gp116 virion envelope protein arises from a common glycosylated precursor of 150 kDa which is further modified by glycosylation into a 160-kDa glycoprotein (Britt, manuscript in preparation) and then cleaved into the mature forms.

The final processing step in the synthesis of mature gp55-116 appeared to be cleavage of the gp160 precursor. The simultaneous generation of two electrophoretically distinct glycoproteins with a combined molecular weight approximating that of the fully glycosylated precursor is consistent with this processing pathway. Several examples of cleavage steps in the processing of viral glycoproteins have been described (9, 11, 13, 15, 16). Recently, Hampl et al. (11) have shown that one group of disulfide-linked envelope glycoproteins of pseudorabies virus is synthesized by cleavage of a higher-molecular-weight polyprotein precursor. Similarly, Little-Van den Hurk et al. (16) have provided extensive information on an envelope protein complex of bovine herpesvirus which was produced by cleavage of a common precursor protein. Finally, proteolytic cleavage of an infected-cell protein of varicella-zoster virus was also shown to be involved in the synthesis of a virion envelope glycoprotein (9). It was interesting that proteolytic cleavage of a larger polyprotein into a disulfide-linked complex of virion envelope glycoproteins is a common synthetic pathway for at least four members of the herpesvirus family (pseudorabies virus, bovine herpesvirus, varicella-zoster virus, and CMV). This feature of herpesviruses may suggest a conserved structural property of the virion envelope in this family of viruses. Definition of such common structural properties of the virion envelope may provide some insight into common functions of these virion proteins in this group of viruses which have very different biological characteristics.

Although our results are consistent with the fact that there is a processing step involving the proteolytic cleavage of the gp160 into the mature gp55-116 complex, other explanations such as in vitro cleavage by cellular protease which is released during cell lysis must be considered. Recent reports have detailed such apparent in vitro artifactual cleavage of herpes simplex virus glycoproteins (19, 28). Although we cannot completely exclude the possibility that release of cellular proteases is responsible for the cleavage of gp160 into the gp55-116 complex, several results are inconsistent with such an explanation. All steps involving cell lysis, immune precipitation, and washing of immune precipitates were carried out at 4°C and in the presence of the protease inhibitor phenylmethylsulfonyl fluoride. In addition, published reports detailing herpes simplex virus glycoprotein cleavage indicate that cellular protease activity is extremely cell line specific (28), and similar protease activity has not been demonstrated in human fibroblasts. Perhaps the most convincing evidence of the intracellular cleavage of gp160 were the nearly identical results obtained when either conventional immune precipitation and SDS-PAGE or Western immunoblotting was used to analyze infected-cell proteins. It can be argued that the prolonged incubation of antibody and cell lysate required for immune precipitation reactions can be predisposed to in vitro proteolysis. However, the preparation of infected-cell proteins for Western immunoblotting, which involved extraction with 5% 2-ME and 2% SDS containing buffer and immediate heating to 100°C, would be expected to destroy most, if not all, proteolytic activity in the cell extracts. Because at least one of the fully processed forms, gp55, was detectable by Western immunoblotting, it was unlikely that in vitro cleavage was responsible for the processing of the gp160 precursor into the mature gp55-116 complex.

The lack of shared antigenicity between gp55 and gp116 of the mature envelope protein complex was somewhat unexpected, as other investigators have suggested that extensive antigenic polymorphism exists among the envelope proteins of other herpesviruses (11, 16), including CMV (17, 20, 21). In fact, a recent report suggests that a single CMV glycoprotein, gA, exists in at least six antigenically related forms in infected cells (21). Because these investigators have not identified the virion forms of these infected-cell proteins, it is difficult to compare their results with our findings which indicate that mature virion gp55 and gp116 do not share antigenic determinants. The failure to detect shared antigenicity may be related to the destruction of antigenic sites during the Western immunoblotting procedure, the use of an insufficient number of monoclonal antibodies to define cross reactivity, or both. However, our results obtained by

using one-dimensional proteolytic peptide mapping of the mature virion forms of these proteins also indicate that gp55 and gp116 are structurally different. One could speculate that these two disulfide-linked proteins may have very different roles in maintaining the structural integrity of the envelope of CMV, in a manner similar to that of the two envelope components of retroviruses (22). Finally, the structural heterogeneity between gp55 and gp116 may also suggest different interactions of these two proteins with the immune response of the host. In this report, as well as in a previous report (3), we have shown that antibodies reactive with gp55 could mediate neutralization of virus in vitro. In addition, antibodies specific for gp55 also detected this protein on the surface of infected cells (Britt, manuscript in preparation). These results suggest that gp55 is expressed on the surface of both infectious virions and infected cells. Thus, a host immune response against gp55 could possibly neutralize infectious virus and eliminate infected cells. Whether gp116 is also expressed on the surface of infectious virions or infected cells remains to be determined.

In summary, we have defined a synthetic pathway for two major envelope glycoproteins of human CMV. These two disulfide-linked glycoproteins, gp55 and gp116, were synthesized as a glycosylated 150-kDa precursor protein which in turn was modified by glycosylation into a 160-kDa precursor polyprotein. gp160 was then cleaved, generating the mature gp55-116 envelope complex. These two mature envelope proteins appear to be antigenically and structurally unique.

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LITERATURE CITED

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