Characterization of a Human Cytomegalovirus Glycoprotein Complex (gcI)

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SUMMARY

Three distinct families of glycoprotein complexes present in the envelopes of human cytomegalovirus and designated gcI, gcII and gcIII have been described recently. The synthesis of the gcI family was analysed using either inhibitors of glycoprotein processing and transport or endoglycosidase treatments of purified glycoproteins. The initial step in gcI synthesis involved the glycosylation of a 95K protein (p95) to form a high-mannose, simple N-linked glycoprotein of M, 158K (gp158), which was detected only in the presence of the glycoprotein processing inhibitor castanospermine. This intermediate was rapidly trimmed in the virus-infected cell to form a more stable simple N-linked precursor glycoprotein of M, 138K (gp138). Treatment of either gp158 or gp138 with endoglycosidase H produced p95. Both molecules, gp158 and gp138, were found in disulphide-linked complexes which are presumably infected cell precursors to gcI since they were not found in virions. The processing of these complexes involved complete cleavage of gp138 and conversion of some but not all of its oligosaccharide to complex N-linked chains. Both processing events were inhibited by the ionophore monensin. Mature gcI contained the gp138 cleavage product, gp55, in a disulphide-linked complex with a heterogeneous glycoprotein designated gp93–130. The latter glycoprotein could be separated into two electrophoretic forms, gp93 and gp130. The deglycosylated form of gp55 had a discrete banding pattern with an apparent Mr of 46K (p46). In contrast, the deglycosylated forms of gp93 and gp130 had diffuse banding patterns with apparent Mr values of 46K to 56K (p46–56) and 60K to 70K (p60–70) respectively. Peptide profiles comparing gp93 with gp130 indicated that they have highly similar polypeptide backbones. Since the deglycosylated forms of gp55 and gp130, 46K and 60K to 70K, respectively, together exceed the 95K precursor/deglycosylated intermediate in Mr, we propose that the above glycoproteins are derived by an alternative proteolytic cleavage of the precursor. The heterogeneous electrophoretic properties of the deglycosylated forms of gp93 and gp130 may be due to additional post-translational modifications other than glycosylation.

INTRODUCTION

Human cytomegalovirus (HCMV), a member of the herpesvirus group, is associated with a wide spectrum of diseases ranging from congenital defects of the newborn to mononucleosis and interstitial pneumonia in immunosuppressed adults (Rapp, 1980; Ho, 1982). Sera from convalescing patients contain antibodies that can neutralize virus infectivity and react with glycoproteins specified by the virus (Pereira et al., 1982a, b; Nowak et al., 1984). Polyclonal antibodies were initially used to define a number of glycoproteins in the infected cell that differed according to molecular weight (Stinski, 1976). Many of these viral glycoproteins were found to reside in the outer envelope of the virion and aberrant particles of HCMV referred to as dense bodies (Sarov & Abady, 1975; Guze, 1976; Kim et al., 1976; Stinski et al., 1979; Gibson,
morphegenesis because antibodies directed against the viral glycoproteins detected the corresponding antigens on the nuclear membranes as well as the internal and external membranes of the cytoplasm of the infected cell (Stinski et al., 1979).

The relationships among HCMV glycoproteins of different molecular weights was not appreciated until monoclonal antibodies were used to define those molecules which share antigenic sites. Pereira et al. (1984) reported a family of antigenically related HCMV glycoproteins designated gA. Cranage et al. (1986) demonstrated that at least one member of this family of glycoproteins had sequence homology to the herpes simplex virus gB gene and, consequently, the gene was referred to as the gB homologue. This gene is located between map coordinates 0.344 and 0.380 for both the Towne (D. R. Gretch & M. F. Stinski, data not shown) and AD169 (Cranage et al., 1986; Mach et al., 1986) strains of HCMV. Earlier studies in the infected cell demonstrated a 95K precursor in the presence of tunicamycin (Pereira et al., 1984).

Pulse–chase studies demonstrated a precursor of 130K to 140K and products of approximately 55K and 93K to 130K (Rasmussen et al., 1985b; Britt & Auger, 1986). Other studies have demonstrated that gp 55 is antigenically related to and derived from gp138 (Britt & Auger, 1986; Mach et al., 1986; Cranage et al., 1986).

Farrar & Greenaway (1986) demonstrated that gp93–130 could be resolved into two discrete electrophoretic species, gp95 and gp130, which were found within different disulphide-linked complexes with gp55. Britt & Auger (1986) concluded that the gp55 and 93K to 130K species were derived from a common precursor but were unrelated to each other structurally and antigenically.

We further investigated the synthesis of gcI in the infected cell. Using the inhibitors castanospermine and monensin as well as endoglycosidases, we demonstrated the existence of two simple N-linked precursor glycoproteins designated gp158 and gp138. These glycoproteins formed disulphide-linked complexes in infected cells which presumably serve as precursor complexes to the gcI family. In addition, we analysed the purified gcI components, gp55, gp93 and gp130, by endoglycosidase treatments and V8 protease peptide mapping.

METHODS

Viruses and tissue culture. The culturing of human foreskin fibroblasts and the propagation of HCMV Towne strain have been described previously (Stinski, 1978). All experiments were conducted at multiplicities of 5 to 10 p.f.u. per ml. To purify virions and dense bodies partially, the tissue culture medium was harvested at 120 h post-infection (p.i.) and passed through a 0.45 µm filter. Virions and dense bodies were centrifuged through 20% D-sorbitol as described previously (Stinski, 1978).

Radioisotope labelling. The labelling of cellular or viral proteins and glycoproteins with [35S]methionine (800 Ci/mmol) or [3H]-sugars (28 to 70 Ci/mmol) (Amersham) has been described (Stinski, 1978). For these studies, radioisotope was used at 50 µCi/ml unless otherwise stated. Pulse labelling with [35S]methionine was performed at 96 h p.i. to minimize background radioactivity in immunoprecipitation experiments.

Inhibitors. Infected cells were pretreated for 4 h with 4.5 µg/ml tunicamycin (Calbiochem), 250 µg/ml castanospermine (Boehringer Mannheim), 1.5 µM-momensin (a gift from C. Grose, University of Iowa) or 2 µg/ml swainsonine (Boehringer Mannheim) in normal medium prior to pulse-labelling for 4 h with 50 µCi/ml of [35S]methionine in the presence of the inhibitor.

Biotinylation of monoclonal antibodies. Monoclonal antibodies were produced as described previously (Kari et al., 1986). For our studies, the prototype monoclonal antibody 41C2, which immunoprecipitates gp55 and gp93–130 (Kari et al., 1986), was used. An ammonium sulphate-enriched fraction of ascites fluid or HPLC-purified monoclonal antibodies at 1 mg/ml was biotinylated as described previously (Gretch et al., 1987).

Immunoprecipitation with biotinylated monoclonal antibodies. Radiolabelled antigen was solubilized in immunoprecipitation buffer [phosphate-buffered saline (PBS) pH 7.4, containing 1% NP40, 0.5% sodium deoxycholate, 0.1% SDS, 100 µg/ml PMSF (Sigma) 0.02% sodium azide] and reacted with 1 to 10 µg of biotinylated monoclonal antibody overnight at 4 °C. The biotinylated antibody–antigen complexes were isolated by incubating the mixture with 50 to 100 µl of streptavidin–agarose (Bethesda Research Laboratories) for 1 h at 4 °C. The streptavidin–agarose containing the biotinylated antibody–antigen complexes was pelleted by centrifugation and then washed four times with PBS pH 7.4, containing 0.1% NP40 and once with H2O. Antigens were eluted with 0.1 M-glycine–HCl pH 2.2, and then titrated to neutral pH with 1 M-NaOH. The antigens were fractionated under
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reducing conditions by electrophoresis in 8\% or 9\% SDS-polyacrylamide gels as described previously (Stinski, 1976).

Endoglycosidase H or F digestion. Endo-\(\beta\)-\(N\)-acetylglucosaminidase H (Endo H) and endo-\(\beta\)-\(N\)-acetylglucosaminidase F (Endo F) (Bethesda Research Laboratories) were used as specified by the manufacturer. Radiolabelled glycoproteins were isolated by immunoprecipitation as described above. The eluted glycoproteins were adjusted to the appropriate salt and detergent conditions for digestion with Endo H or F (0.1 M-sodium phosphate pH 6.1, containing 1\% NP40, 0.1\% SDS and 100 \(\mu\)g/ml PMSF plus 1\% 2-mercaptoethanol and 50 \(\mu\)M-EDTA for the Endo F reaction only). Samples were heated at 100 °C for 5 min, cooled, and 72 \(\times\) 10\(^{-3}\) units of Endo H per ml or 15 units of Endo F per ml were added. Enzyme was omitted from the mock-digested controls. After 18 h at 37 °C, the samples were analysed on 9\% SDS-polyacrylamide gels containing 1\% NP40. Radiolabelled proteins and glycoproteins were detected by autoradiography.

Electro-elution of glycoproteins from SDS-polyacrylamide gels. The electro-elution of radiolabelled glycoproteins from SDS-polyacrylamide gels was performed essentially as described by Farrar & Greenaway (1986). The eluted glycoproteins were dialysed extensively against 1 mM-Tris-HCl pH 8.0 and lyophilized. The glycoprotein residue was solubilized in boiling Endo F buffer prior to further treatment.

Sequential digestion with Endo F, neuraminidase and \(O\)-glycanase. Solubilized glycoproteins were digested with Endo F as described above except that fresh enzyme was added after an initial 10 h treatment, and the total treatment time was 20 h. After Endo F treatment, samples were heated at 100 °C for 5 min, cooled, and subjected to 2 h digestions twice with 30 \(\times\) 10\(^{-3}\) units neuraminidase (Sigma) at 37 °C. The samples were heated at 100 °C for 5 min, cooled and subjected to 10 h digestions twice with 7.5 \(\times\) 10\(^{-3}\) units endo-\(\alpha\)-\(N\)-acyetyl-\(D\)-galactosaminidase (\(O\)-glycanase; Genzyme, Boston, Mass., U.S.A.) at 37 °C. Enzyme was omitted from mock-digested controls. The digested and mock-digested samples were analysed on 8\% SDS-polyacrylamide gels as described above.

Peptide profile. Radiolabelled glycoproteins were isolated by immunoprecipitation and SDS-PAGE. Endo F, neuraminidase and \(O\)-glycanase-treated or untreated glycoproteins were fractionated by SDS-PAGE and detected by autoradiography. Radioactive bands were cut from dried gels and treated as described by Cleveland et al. (1977). Protein digestion was allowed to proceed for 30 min in the presence of 60 \(\mu\)g of staphylococcal V8 protease. The resulting peptides were fractionated in 15\% SDS-polyacrylamide gels and analysed by autoradiography.

RESULTS

Effect of tunicamycin, castanospermine, monensin or swainsonine

To characterize precursor/product relationships, a series of inhibitors of glycoprotein processing were used. The antibiotic tunicamycin blocks the initial addition of high-mannose carbohydrate moieties to peptide backbones (Takatsuki et al., 1975; Leavitt et al., 1977). Castanospermine blocks the removal of terminal glucose residues from high-mannose glycoproteins (Elbein, 1984). The ionophore monensin prevents the transport of glycoproteins from the \(cis\) face to the \(trans\) face of the Golgi apparatus (Johnson & Spear, 1982) and, consequently, later glycosylation events are inhibited (Srinivas et al., 1982). Swainsonine inhibits Golgi apparatus mannosidase II and, consequently, some late glycoprotein processing events can not occur (Elbein et al., 1982; Elbein, 1984).

Cell cultures at 96 h p.i. were either untreated or pretreated with inhibitor for 4 h and then pulse-labelled with \([35S]\)methionine for 4 h in the presence of inhibitor. Glycoproteins were solubilized and immunoprecipitated as described in Methods. Monoclonal antibody 41C2 immunoprecipitated three glycoproteins designated gp138, gp93–130 and gp55 from untreated cells (Fig. 1a, Fig. 1b, lane 2). However, only gp93–130 and gp55 were found to be associated with purified virions and dense bodies (Fig. 1a, lane 6). Using other radioactive labels including \([35S]\)cysteine, \([3H]\)glucosamine and \([3H]\)mannose produced identical results (data not shown). In the presence of tunicamycin, the predominant immunoprecipitated band was 95K, although some gp138 and two non-glycosylated bands at 200K and 64K were also present (Fig. 1a, lane 3). The 200K protein was occasionally detected in mock-infected controls (data not shown), while the 64K band is probably the virion tegument protein. In the presence of castanospermine, an intermediate glycoprotein of 158K was observed (Fig. 1b, lane 3), as well as two other bands which migrated similarly to gp93–130 and gp55. In the presence of monensin, there was a marked accumulation of the precursor glycoprotein gp138 but gp93–130 and gp55 were not detected (Fig. 1a, lane 4). When castanospermine and monensin were present simultaneously, only the 158K intermediate accumulated (Fig. 1b, lane 4). Swainsonine caused a marginal
increase in the mobility of gp93–130 and gp55 (Fig. 1a, lane 5). These results suggest the following. (i) The nascent polypeptide backbone is a protein of 95K. This is consistent with earlier observations (Pereira et al., 1984; Cranage et al., 1986). (ii) The precursor high-mannose form is a 158K intermediate glycoprotein which we designated gp158. In the presence of castanospermine, some of the high-mannose form may be processed to products which resemble gp93–130 and gp55. This processing is blocked by monensin. (iii) Monensin causes an accumulation of gp138 and blocks the production of the mature forms of gcI, gp93–130 and gp55. This suggests that the cleavage of gp138 takes place in the Golgi apparatus, since monensin is known to block transport within this organelle (Johnson & Spear, 1982). (iv) Swainsonine had a minor effect on the oligosaccharide processing of gp93–130 and gp55, because in the presence of the drug these two glycoprotein species migrated slightly faster. (v) The precursor glycoproteins, gp158 and gp138, are not incorporated into virions or dense bodies.

**The precursor glycoproteins form disulphide-linked complexes prior to cleavage**

To determine whether the glycoprotein precursors gp158 and gp138 were present in the infected cell as monomers or disulphide-linked complexes, infected cells were treated with monensin or monensin plus castanospermine, pulse-labelled, and immunoprecipitated as described above. gp138 and gp158 were detected as complexes as well as monomers when fractionated by non-reducing SDS–PAGE (Fig. 2a). In addition, these species migrated as high molecular weight complexes when analysed by rate zonal centrifugation (data not shown). Only
monomers were detected by reducing SDS–PAGE (Fig. 2b). Thus, the precursors gp158 and gp138 were forming disulphide-linked complexes prior to further processing. These complexes presumably serve as precursors to the mature virion-associated gcl family.

Endoglycosidase digestions

To analyse the nature of the carbohydrate modifications, the above glycoprotein species were digested with either Endo H, Endo F or a combination of Endo F, neuraminidase and O-glycanase. Our goal was to reduce the above glycoproteins to their deglycosylated forms to facilitate comparison of the individual species. Endo H cleaves specifically simple N-linked carbohydrate chains while Endo F cleaves both simple and complex N-linked oligosaccharides (Tarentiro & Maley, 1974; Tai et al., 1977). O-glycanase cleaves specifically O-linked carbohydrate chains (Takasaki & Kobata, 1976).

The glycoproteins were radiolabelled with [35S]methionine, immunoprecipitated with monoclonal antibody 41C2 and gel-purified as described in Methods. gp 158 was radiolabelled in the presence of castanospermine and monensin. gp138 was radiolabelled in the presence of monensin. Treatment of gp158 or gp138 with Endo H produced a 95K species (Fig. 3a and 3b,
Fig. 3. Endoglycosidase H and F treatment of gp158, gp138 and gp55. HCMV-infected cells were pulsed from 92 to 96 h p.i. with 200 μCi of $^{35}$S-methionine in the presence of monensin, castanospermine plus monensin or no inhibitor as described in the legend of Fig. 1. Radiolabelled antigens were immunoprecipitated with monoclonal antibody 41C2 and digested with Endo H or Endo F. Individual glycoprotein species were gel-purified as described in the text. (a) Gel-purified gp158 treated with (lane 2) or without (lane 1) Endo H. Lane 3, Mr standards. (b) Gel-purified gp138 treated with (lane 2) or without (lane 1) Endo H. (c) Mixture of gp138 and gp55 treated with (lane 2) or without (lane 1) Endo H. (d) Gel-purified gp55 treated with (lane 2) or without (lane 1) Endo F.

respectively). When $[^3H]$mannose-labelled gp138 was digested with Endo H, the radioactive band disappeared, indicating that all carbohydrate had been removed (data not shown). Treatment of a mixture of gp138 and gp55 with Endo H produced the 95K species and a glycosylated form of 50K (Fig. 3c). gp50 retained some $[^3H]$mannose label after digestion with Endo H (data not shown). When $^{35}$S-methionine-labelled gp55 was treated with Endo F, it migrated as a 46K protein (Fig. 3d).

These results indicated that gp158 and gp138 are simple N-linked glycoproteins with a peptide backbone of 95K. This is consistent with the observed effect of tunicamycin (see Fig. 1a, lane 3). The high-mannose form is gp158 since it accumulated in the presence of castanospermine (see Fig. 1b, lane 3). These results also suggest that gp55 contains approximately 5K of simple N-linked carbohydrate, approximately 4K of complex N-linked carbohydrate and a peptide backbone of 46K.

Farrar & Greenaway (1986) demonstrated that the diffuse glycoprotein gp93–130 could be resolved into two distinct electrophoretic forms, designated gp95 and gp130. Therefore, we resolved gp93–130 into two electrophoretic forms to study their carbohydrate modifications and polypeptide backbones. gp55 was purified as a control. The gel-isolated glycoprotein species were dialysed, lyophilized, and then extensively digested twice with Endo F followed by neuraminidase twice and finally O-glycanase twice as described in Methods. The results of sequentially digested as well as mock-treated gp55, gp93 and gp130 are presented in Fig. 4.

Treatment of gp55 (Fig. 4, lane 2) with Endo F, neuraminidase and O-glycanase produced a 46K protein (Fig. 4, lane 3). This result was identical to that of Endo F treatment alone (see Fig. 3d). Sequential treatment of gp93 (Fig. 4, lane 4) with the three enzymes produced a species with
Fig. 4. Sequential digestion of gel-purified gp55, gp93 and gp130 with Endo F, neuraminidase and O-glycanase. Infected cells were pulse-labelled with [35S]methionine, virions and dense bodies were purified. After immunoprecipitation, gp55, gp93 and gp130 were separated in an 8% SDS-polyacrylamide gel and recovered by electro-elution as described in the text. The recovered glycoproteins were sequentially digested or mock-digested with Endo F twice for 20 h followed by neuraminidase twice for 4 h and O-glycanase twice for 20 h, as described in the text. The digested and mock-digested glycoproteins were analysed by electrophoresis in an 8% SDS-polyacrylamide gel under reducing conditions. Lanes 1 and 8, 14C-labelled Mr standards; lane 2, gp55 electro-eluted and mock-digested; lane 3, gp55 electro-eluted and sequentially digested; lane 4, gp93 electro-eluted and mock-digested; lane 5, gp93 electro-eluted and sequentially digested; lane 6, gp130 electro-eluted and mock-digested; lane 7, gp130 electro-eluted and sequentially digested. The Mr values of the protein standards, glycoproteins (gp) and digested glycoproteins are designated.

an Mr of 46K to 56K (Fig. 4, lane 5). This shift in Mr was considerably greater than that observed after gp93–130 had been treated with Endo F alone (data not shown). Sequential treatment of gp130 (Fig. 4, lane 6) with the three enzymes produced a diffusely migrating species with an Mr range of 60K to 70K (Fig. 4, lane 7). Homogeneously migrating peptides were not detected after extensive sequential digestion of gp93 and gp130. There are several possible reasons for this. First, these species may be composed of multiple polypeptide backbones, possibly due to aberrant cleavage during processing or multiple gene products. Second, the species may contain endoglycosidase-resistant sugars, particularly of the O-linked branched chain variety (Lamblin et al., 1984). Third, other protein modifications such as sulphation, fatty acylation or phosphorylation may contribute to the observed heterogeneity (Montalvo & Grose, 1987). Our experiments suggest that only N-linked sugars are present in gp55, both N- and O-linked sugars are present in gp93 and gp130, and that the post-translation processing of the polypeptide backbones of gp93 and gp130 is considerably different from that of gp55.

Peptide profiles of gp93 and gp130

To understand better the relationships between gp93 and gp130, we compared V8 protease digestion profiles by the method of Cleveland et al. (1977). These two electrophoretic forms were purified as described for Fig. 4, digested with V8 protease, and analysed (Fig. 5a). Their peptide
Fig. 5. Peptide profiles of the glycosylated and deglycosylated gp93 (a, lane 2) and gp130 (a, lane 1). Radiolabelled glycoproteins or deglycosylated proteins were prepared and fractionated in 8% SDS-polyacrylamide gels. The glycoproteins were electro-eluted and mock-digested or sequentially digested with Endo F, neuraminidase and O-glycanase as described for Fig. 4. Glycoproteins or deglycosylated proteins were fractionated by SDS-PAGE, located by autoradiography, cut from the gels and digested with 1.2 μg of staphylococcal V8 protease. The digested proteins and glycoproteins were fractionated on 15% SDS-polyacrylamide gels and the proteolytic digestion profiles were analysed by autoradiography as described in the text. (b) lane 1, p60-70; lane 2, p60-70; lane 3, p46-56. Arrows designate peptides or glycopeptides with similar electrophoretic mobilities.

profiles were highly similar if not identical, as the arrows in Fig. 5a indicate. Next, purified gp93 and gp130 were digested extensively with Endo F, neuraminidase and O-glycanase as described for Fig. 4. The species designated p60-70 and p46-56 were generated from gp130 and gp93, respectively, and digested with V8 protease. The peptide profiles of p60-70 and p46-56 were also similar (Fig. 5b). The diffuse migrating bands generated after deglycosylation presumably represent different degrees of post-translational modification which is related to the very diffuse electrophoretic properties of gp93 and gp130. Peptide profile comparisons between gp55, gp93 and gp130 as well as their deglycosylated forms did not render clear and unequivocal results. Their polypeptide profiles had differences as well as similarities (data not shown) and, therefore, alternative methods for peptide analysis such as N-terminal sequencing and two-dimensional peptide mapping are required.

DISCUSSION

There have been numerous studies of HCMV glycoproteins since they were first described (Stinski, 1976), yet confusion remains concerning the synthesis and processing of these immunologically important molecules. Our current study has focused on the synthesis and
processing of a family of glycoproteins which was originally designated gA by Pereira et al. (1984) and mapped by Mach et al. (1986). However, the gene that codes for at least one member of the family, gp55, has homology with the herpes simplex virus gB gene (Cranage et al., 1986) and consequently, this glycoprotein is also referred to as the gB homologue. In the infected cell three glycosylated species referred to here as gp138, gp93–130 and gp55 have been observed previously (Rasmussen et al., 1985b; Britt & Auger, 1986). In our current study, we demonstrated a fourth species, the high-mannose intermediate gp158, which accumulated only in the presence of the processing inhibitor castanospermine. This species is rapidly trimmed in infected cells to form the stable precursor gp138. Both gp158 and gp138 have 95K polypeptide backbones and contain exclusively simple N-linked sugars. In addition, both glycoproteins were found in disulphide-linked complexes in infected cells but not virions. Thus, these complexes may be precursors to the mature virion-associated gcI.

The processing of the precursor complexes to mature gcI involves the cleavage of gp138 to at least one product, gp55, which contains both simple and complex N-linked oligosaccharides and a 46K polypeptide backbone. The cleavage of gp138 and production of gp55 was inhibited by the ionophore monensin. In the virion envelope, gp55 was found only in disulphide-linked complexes (the gcI family) which also contained a heterogeneous glycoprotein designated gp93–130. The latter species was found in relatively low abundance in infected cells but is present at relatively high levels in virion envelopes (Farrar & Oram, 1984). Farrar & Greenaway (1986) demonstrated that this species could be separated into two distinct electrophoretic forms. These forms, designated gp95 and gp130, were distributed unevenly in the virion complexes. Our current study indicates that these two forms contain highly related polypeptide backbones. In addition, they contain both complex N- and O-linked oligosaccharide chains as well as fucose and sialic acid (B. Kari, personal communication). The molecular basis for the different electrophoretic mobilities and distribution of gp93 and gp130 within the gcI is not understood at present.

The relationship of gp93–130 to gp55 is also a controversial issue. Britt & Auger (1986) proposed that these two molecules are derived from completely different areas of the 95K precursor and, therefore, are structurally and antigenically different. However, gp55-specific antisera occasionally reacted with virion proteins of 96K, 130K and 160K (Mach et al., 1986). These results suggest that gp55 and gp93–130 may contain common epitopes. The data in this manuscript and immunological data (Kari et al., 1986) suggest that gp93 and gp130 may be derived from the gB gene. Peptide mapping experiments suggested that gp93 and gp130 contained protein domains in common with gp55 as well as unique protein domains (D. R. Gretch & M. F. Stinski, unpublished data). Since the deglycosylated forms of gp55 and gp130, 46K and 60K to 70K, respectively, together exceed in size the precursor polypeptide backbone of 95K, it is possible that these glycoproteins are generated by differential cleavage of the gp138 precursor. Alternatively, there might be differential splicing of the gB mRNA. The first explanation is more likely because our inhibitor studies identified only one precursor polypeptide and one high-mannose intermediate. Since the gB open reading frame predicts as many as seven dibasic protease recognition sites (Cranage et al., 1986), alternative cleavage of the gp138 precursor may better explain the gp55, gp93 and gp130 products of the gB gene.

Our data indicate that the polypeptide backbones of gp93 and gp130 differ considerably in electrophoretic behavior from that of gp55. The heterogeneous electrophoretic behaviour of gp93 and gp130 is presumably due to some additional post-translational modification such as phosphorylation or sulphation, or incomplete removal of carbohydrate residues. Since gcI is a major component of the outer virion envelope (Gretch et al., 1988), it is almost certainly important in the host immune response to HCMV infection. Studies with HCMV (Rasmussen et al., 1985a; Gönçzöl et al., 1986) and other herpesviruses, such as herpes simplex virus (Cohen et al., 1978; Spear, 1980; Norrild, 1980), Epstein–Barr virus (Thorley-Lawson & Geilinger, 1980; North et al., 1982), and varicella-zoster virus (Grose et al., 1984) have demonstrated that specific virion envelope glycoproteins are important for determining the host’s immune response to virus infection. Further study of gcI as an immunogen in vivo and in vitro should facilitate our understanding of humoral as well as cellular immunity to HCMV infection.
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HC MV glycoprotein complex


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