Characterization of Glycoprotein Complexes Present in Human Cytomegalovirus Envelopes

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SUMMARY

Three disulphide cross-bridged glycoprotein complexes were immunoprecipitated from purified human cytomegalovirus envelopes using a monoclonal antibody with a specificity for a glycoprotein of mol. wt. 52 × 10³. These complexes were isolated by electroelution after polyacrylamide gel electrophoresis (PAGE) under non-reducing conditions. Compositional analysis of each complex by PAGE under reducing conditions showed that at least two distinct complexes, one containing glycoproteins with mol. wt. of 52 × 10³ and 95 × 10³ and the other with glycoproteins of 52 × 10³ and 130 × 10³, were present. The results obtained indicated that one of these complexes could also exist as a dimer.

The human cytomegalovirus (HCMV) genome codes for a number of glycoproteins which ultimately reside in the outer envelope of the mature virion (Stinski, 1976; Farrar & Oram, 1984). Although these glycoproteins are not the most abundant virion components they have been reported to elicit antibodies that neutralize virus infection in vitro (Pereira et al., 1982a, b; Britt, 1984). Monoclonal antibodies produced against the HCMV glycoproteins have been used to classify these proteins into groups that share antigenic sites (Pereira et al., 1982b; Nowak et al., 1984) or which have precursor/product relationships (Pereira et al., 1984). However, the structural and functional relationships existing between the various glycoprotein species within the virion envelope require further investigation as it is these which may ultimately determine the interactions between the virus and susceptible cells and between the virus and the host immune system. High molecular weight HCMV envelope glycoprotein complexes have been reported by Britt (1984) and, more recently, by Law et al. (1985). In this paper we extend these studies and describe the co-existence of three complexes consisting of different glycoprotein subunits.

The production of HCMV strain AD169 in human foetal foreskin fibroblast monolayers, the subsequent preparation of purified virions by centrifugation through glycerol–tartrate gradients (Talbot & Almeida, 1977) and electrophoretic separation of purified virion components have been described previously (Farrar & Oram, 1984). Purified virus was labelled with ¹²⁵I by the method of Hunter & Greenwood (1962). Unincorporated isotope was removed by repeated centrifugation and resuspension of the pellet in phosphate-buffered saline (PBS). Virion envelope components were extracted from pelleted virus particles by dispersion in 500 μl of 10 mm-Tris–HCl, 1·0 mm-CaCl₂, 0·15 M-NaCl, 1% Triton X-100, 2 mm-phenylmethylsulphonyl fluoride, 1% ethanol, pH 7·3 (buffer A) and subsequent treatment in an ultrasonic water-bath (4 × 15 s at 0 °C). After 20 min at 0 °C the turbid suspension was centrifuged at 20000 r.p.m. (Sorvall SS34 rotor) for 1 h at 0 °C.

Solubilized components were immunocomplexed using a monoclonal antibody specific for a glycoprotein of mol. wt. 52 × 10³ (gp52) (Law et al., 1985). Non-reacting components were removed, after attachment of the immunocomplexes to Protein A–Sepharose, by subsequent washing of the beads in PBS. Glycoprotein complexes were released from the Protein A–
Fig. 1. Autoradiographs of $^{125}$I-labelled HCMV polypeptides. (a) Whole purified virion and (b) immunoprecipitated components separated under reducing (lanes 1) and non-reducing (lane 2) conditions. Polypeptides were separated by SDS-PAGE in 7.5% gels. Numbers refer to mol. wt. ($\times 10^3$).

Sepharose beads and the monoclonal antibody by incubation with 0.0625 M-Tris-HCl, 1% SDS pH 6.2 (buffer B) or buffer B containing 5% (v/v) 2-mercaptoethanol for 3 min at 100 °C. The solubilized samples were electrophoresed in linear polyacrylamide gels in the presence of SDS (0.1%, w/v) as described by Laemmli (1970). The gels were dried after electrophoresis and then exposed with Cronex L X-ray film (Dupont) at −80 °C.

Fig. 1(a) shows the composition of purified HCMV virions and Fig. 1(b) demonstrates the results obtained after electrophoresis of the immunoprecipitated complexes under reducing (lane 1) and non-reducing (lane 2) conditions. In the presence of 2-mercaptoethanol (a reducing agent), glycoproteins with mol. wt. of $52 \times 10^3$, $95 \times 10^3$ and $130 \times 10^3$ (gp52, gp95 and gp130; Farrar & Oram, 1984) were released from the immunoprecipitated components. In the absence of 2-mercaptoethanol, complexes with mol. wt. of $150 \times 10^3$, $180 \times 10^3$ and approximately $250 \times 10^3$ (complexes 1 to 3 respectively; Fig. 1b, lane 2) were observed. These complexes were subsequently separated by preparative gel electrophoresis in a linear 5% acrylamide gel. The
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Fig. 2. Autoradiographs of $^{125}$I-labelled HCMV envelope glycoprotein complexes and their constituent glycoproteins. Mixtures of glycoprotein complexes 1 to 3 were immunoprecipitated using a monoclonal antibody with a specificity for gp52 and separated under non-reducing conditions (a). Individual glycoprotein complexes (lanes 1 to 3 respectively) were examined under non-reducing (b) and reducing (c) conditions. Native and reduced complexes were separated by SDS-PAGE in 5% (a) and 7.5% (b, c) acrylamide gels and autoradiographed as unfixed and wet (a) or fixed and dried (b, c) gels. Numbers refer to mol. wt. ($\times 10^3$).

gels were sealed between plastic sheets after electrophoresis and placed in contact with X-ray film at 4°F for 12 h. The developed autoradiogram (Fig. 2a) was used to locate each of the complexes which were then separately electroeluted as described by Greenaway & LeVine (1973). Electroeluted material was dialysed against 0.05 M-ammonium bicarbonate (5000 vol.) for 12 h at 4°F and lyophilized. The freeze-dried material was redissolved in a minimum volume of buffer B and the separated complexes were analysed by electrophoresis on 7.5% acrylamide gels (Fig. 2b).

The individual glycoprotein complexes in buffer B were reduced by the addition of 2-mercaptoethanol to a final concentration of 5% (v/v) and boiled for 3 min at 100°F prior to electrophoresis on 7.5% linear gels as described above. The composition of each complex is shown in Fig. 2(c). The recovery of glycoprotein complexes 1 to 3 by electroelution was 80 to 90% efficient as measured by radioactivity. The demonstration that all three complexes contained gp52 (Fig. 2c) is consistent with the previous observation that the immunoprecipitating monoclonal antibody is specific for this protein (Law et al., 1985).

From the data shown in Fig. 2(c) it is clear that the abundant HCMV envelope glycoproteins gp95 and gp130 exist in discrete assemblies with gp52. As the molecular weights of complexes 1 and 2 are approximately $150 \times 10^3$ and $180 \times 10^3$ respectively (Fig. 2b), we propose that in these, one molecule of gp52 is associated with one molecule of either gp95 or gp130. Complex 3 which also contains gp52 and gp95 and which has an approximate mol. wt. of $250 \times 10^3$ (Fig. 2b), may represent a dimer of complex 1. The minor high molecular weight components present in lanes 1 and 2 of Fig. 2 probably represent unreduced parental complexes.
Britt (1984) and Rasmussen et al. (1985) have both observed high molecular weight complexes within extracts of purified HCMV. The conditions of solubilization, the molecular weights and the compositions of these complexes differ significantly from each other and from those reported here. Britt (1984) observed a single complex of mol. wt. approximately $300 \times 10^3$ consisting of three protein species of size 55, 116 and 160, all $\times 10^3$. In contrast, Rasmussen et al. (1985), using ionic detergents, solubilized a complex of approximate molecular weight $200 \times 10^3$ comprising a single protein with mol. wt. $55 \times 10^3$. The constituents of these complexes were, like those in the complexes described here, held together by reduction-sensitive disulphide cross-bridges. We have now shown that at least two bimolecular glycoprotein complexes exist in the HCMV envelope and that one of these may be able to dimerize. We therefore conclude that these virion envelope complexes differ from those reported by Britt (1984) and Rasmussen et al. (1985).

Neutralizing and non-neutralizing monoclonal antibodies with specificities for different HCMV glycoproteins tend to recognize more than one component by immunoprecipitation (Pereira et al., 1982b, 1984; Nowak et al., 1984; Britt, 1984; Law et al., 1985). Interestingly, the molecular weights of several groups of these co-precipitating glycoproteins are in good agreement with those for the components of the envelope complexes reported here. The co-precipitation of different glycoproteins by the same monoclonal antibody may therefore result from associations through disulphide bonds (Britt, 1984; Law et al., 1985) rather than from the occurrence of either common antigenic determinants (Nowak et al., 1984) or precursor/product relationships (Pereira et al., 1984).

Human convalescent sera recognize many HCMV polypeptides, including glycoproteins 52, 95 and 130 (Pereira et al., 1982a; Middledorp et al., 1985) and mixtures of HCMV envelope components have been shown to elicit humoral and cellular immunity in experimental animals (Furukawa et al., 1984). In addition, envelope glycoproteins from purified HCMV virions, including those present in the complexes described here, are immunogenic (Farrar & Oram, 1984). However, the role played by individual glycoprotein species or complexes in the prevention and limitation of HCMV-associated disease is unknown. Nevertheless, similar glycoproteins in other herpesviruses appear to fulfil this role. For example, well defined amino acid sequences of the herpes simplex virus glycoprotein D are known to mimic the ability of the intact protein species to elicit neutralizing antibodies (Eisenberg et al., 1985).

The immunogenicity of the isolated glycoproteins is relatively low compared with the intact virus, suggesting that a cooperative interaction between these proteins is important in determining an efficient immune response. Alternatively, three-dimensional conformations involving one or more glycoproteins may be important for the expression of effective neutralizing epitopes. Indeed, the conformation of the Epstein–Barr virus membrane antigen, gp340, is crucial in determining its capacity to elicit potent neutralizing antibodies (North et al., 1982). Clearly our understanding of the interactions between the HCMV envelope and the host immune system will be greatly enhanced by comparison with the situation known to exist with other herpesviruses and by the availability of the different complexes and individual glycoprotein components in a purified form for structural and immunological studies.

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REFERENCES


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