Identification of the gB Homologues of Equine Herpesvirus Types 1 and 4 as Disulphide-linked Heterodimers and Their Characterization Using Monoclonal Antibodies

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SUMMARY
Equine herpesvirus types 1 and 4 (EHV-1 and EHV-4) labelled with $[^{14}\text{C}]$glucosamine were purified from infected cell culture medium and profiles of their structural proteins were obtained that enabled identification of the major glycoproteins. Nine glycosylated polypeptides were identified for each virus. Preparations of the purified viruses each contained a glycoprotein which was linked by disulphide bonds, as determined by diagonal gel electrophoresis under reducing/non-reducing conditions. High $M_t$ forms of this glycoprotein were detected for EHV-1 when the sample was not heated. The EHV-1 protein consisted of three polypeptides of $M_t$ 108K, 76K and 58K and the EHV-4 protein consisted of three polypeptides of $M_t$ 112K, 74K and 61K. Western blotting and immunoprecipitation with monoclonal antibodies confirmed that the EHV-1 gB homologue migrates with an apparent $M_t$ of 108K (140K under non-reducing conditions) but is cleaved to give glycoproteins of 76K and 58K which are held together by disulphide bonds. The EHV-4 gB homologue consists of a 112K glycoprotein which is cleaved to give glycoproteins of 74K and 61K which are also linked by disulphide bonds.

INTRODUCTION
Equine herpesvirus type 1 (EHV-1) is a major respiratory pathogen of horses, and causes abortion and paralysis in pregnant mares (O’Callaghan et al., 1983). It has now become clear that there are two distinct subtypes, 1 and 2, demonstrated by restriction endonuclease digestion of purified virus DNA (Sabine et al., 1981; Studdert et al., 1981; Allen & Turtinen, 1982). EHV-1 subtype 1 is responsible for respiratory disease, abortion and paralysis, whereas the subtype 2 appears to cause only rhinopneumonitis (Allen & Bryans, 1986). Further analysis of the genome structure of these two viruses has now led to the suggestion that they be renamed EHV-1 and EHV-4, the former being the abortifacient virus and the latter being the respiratory type.

Early experiments on the structural proteins of EHV-1 were limited to high-passage tissue culture adapted strains (Perdue et al., 1974) or hamster-adapted strains (Kemp et al., 1974). A more recent paper has reported the presence of up to 12 glycoproteins in the envelope of purified virus (Turtinen & Allen, 1982) and another has compared, from purified preparations, the polypeptide profiles of EHV-1 and EHV-4 (Turtinen et al., 1981). The role of the structural proteins and the degree of similarity between the two viruses still remains unclear.

Allen & Yeargan (1987) have recently reported the mapping of six separate genes which code for EHV-1 glycoproteins by using a panel of monoclonal antibodies and a $\lambda gt$ 11 expression system. Two genes were provisionally assigned as homologous with herpes simplex virus type 1 (HSV-1) gB and gC, based on their apparent collinearity on the virus genome. There are no equivalent data available for EHV-4.
We have previously established that three polypeptides (138K, 100K, 87K) of EHV-1 cross-react with a polyclonal antiserum raised against HSV-1 gB (Snowden et al., 1985) and that at least one of these polypeptides (138K) has a tryptic peptide profile similar to that of HSV-1 gB. The \( M_r \) of the EHV-1 protein, shown by Allen & Yeargan (1987) to be collinear with HSV-1 gB, is 87K which may correspond to one of the polypeptides already identified in this laboratory (Snowden et al., 1985). It is now becoming clear that the envelope glycoproteins of many herpesviruses which share homology with HSV-1 gB are disulphide-linked heterodimers (Marshall et al., 1986; Lukács et al., 1985; Keller et al., 1986; Cranage et al., 1986) and the analyses of the amino acid sequences deduced from the DNA sequences reveal putative proteolytic cleavage sites in the primary amino acid sequences at a position which would produce two polypeptides of the \( M_r \) values predicted by SDS–PAGE. N-terminal amino acid sequence data have been obtained for the polypeptides of varicella-zoster virus gB (Keller et al., 1986) and the sequences obtained match those predicted from the nucleotide sequence, thus confirming that the two polypeptides were products of a single gene.

The experiments reported here aim to identify the major structural glycoproteins of characterized strains of EHV-1 and EHV-4 and to show that the gB homologues of EHV-1 and EHV-4 exist as disulphide-linked heterodimers.

### METHODS

**Cells and media.** RK13 cells were grown in the autoclavable form of the Glasgow modification of Eagle's medium (Flow Laboratories) containing 5% calf serum (EC5) and equine dermal cells (NBL-6, Flow Laboratories) were grown in Dulbecco's modification of Eagle's medium supplemented with 10% foetal calf serum (DMEM 10).

**Viruses and virus assays.** Virus strains used were EHV-1 strain Ab1, a low passage isolate from a case of abortion at Wood Ditton Stud and EHV-4 strain MD, a lung isolate of low passage; both were kindly provided by Dr J. Mumford, Equine Virology Unit, Newmarket, U.K. Stocks of EHV-1 were grown in RK13 cells, those of EHV-4 were grown in NBL-6 cells. Plaque assays were performed by the method of Russell (1962), using an overlay medium containing carboxymethyl cellulose.

**Virus purification.** EHV-1 and EHV-4 were purified from the culture medium of RK13 and NBL-6 cells respectively infected with virus at 0.1 p.f.u./cell and harvested 48 to 72 h after infection. Virus was concentrated from the extracellular medium by centrifugation at 15 000 g for 90 min at 4 °C, resuspended in a minimum volume of phosphate-buffered saline (PBS) and layered onto preformed gradients of 10% to 40% (w/v) sucrose in PBS. Gradients were centrifuged at 30 000 g for 1 h, the virus band was aspirated and diluted fourfold with PBS. Virus was then concentrated by centrifugation at 80 000 g, resuspended in water and stored at −70 °C until use. \([^{14}C]\)Glucosamine-labelled virus preparations were isolated in a similar manner except that infected cells were incubated for at least 16 h after infection in culture medium containing half the normal concentration of glucose and 0.2 μCi \([^{14}C]\)glucosamine/ml (50 mCi/mmol; Amersham).

**SDS–PAGE.** Gels cross-linked with NN'-diallyltartardiamide (DATD), were prepared by standard techniques (Heine et al., 1974) with a final concentration of 9% acrylamide. Prior to electrophoresis, protein samples were solubilized by boiling (unless otherwise indicated) in gel loading buffer containing 0.125 M-Tris–HCl pH 6.8, 2% (w/v) SDS, 5 mM-dithiothreitol (DTT), 0.1% (w/v) bromophenol blue and 10% (v/v) glycerol. \( M_r \) standards ranged from 205K (myosin) to 43K (ovalbumin) (Bio-Rad). Two-dimensional (diagonal) gel electrophoresis in the presence and absence of reducing agent in the first dimension was performed by the method of Zweig et al. (1979), except that 9% DATD cross-linked gels were used in both dimensions. Tube gels which were not reduced during electrophoresis in the first dimension were soaked in 50 mM-Tris–HCl pH 6.8, 0.1% SDS and 10 mM-DTT for 30 min at room temperature, prior to electrophoresis in the second dimension.

**Fluorography.** Polyacrylamide gels were prepared for fluorography by treatment with 2,5-diphenyloxazole as described by Laskey & Mills (1975).

**Protein iodinations.** Preparations of purified virus were suspended in 0.2 M-borate buffer pH 8.5, 1% NP40 and treated with 25 μCi of \([^{125}I]\)-labelled Bolton and Hunter reagent redissolved in anhydrous propanol, for 1 h at 0 °C. The reaction was terminated by the addition of an equal volume of 0.2 M-glycine and the mixture incubated at 0 °C for a further 15 min. For immune precipitation, low \( M_r \) iodinated species were separated from labelled proteins by gel filtration of the sample on a 1 ml column of Sephadex G-25 (Pharmacia) equilibrated in RIPA buffer (1% w/v NP40, 0.1% w/v SDS, 1% w/v deoxycholate in PBS) containing 0.1% gelatin and the insoluble material was removed by centrifugation of the sample at 12 500 g for 5 min.

**Monoclonal antibodies.** Murine monoclonal antibodies to EHV-1 were prepared by inoculation of BALB/c mice with purified EHV-1 by the method of Killington et al. (1981). Positive antibody-secreting clones were identified by screening the culture supernatants by ELISA using purified virus as the antigen source and then cloned by
**EHV-1 and EHV-4 gB homologue**

Fig. 1. Coomassie Brilliant Blue R-250-stained SDS–PAGE profiles of purified preparations of EHV-1 Ab1 (lane 1) and EHV-4 MD (lane 2) on 9% polyacrylamide gels. The $M_r$ values of major polypeptides are indicated ($\times 10^3$) and were calculated using $M_r$ standards of myosin (205K), $\beta$-galactosidase (116K), phosphorylase (97K), bovine serum albumin (67K) and ovalbumin (43K).

Fig. 2. SDS–PAGE analysis on 9% polyacrylamide gels of Ab1 (lanes 1 and 2) and MD (lanes 3 and 4), labelled with $^{[35S]}$methionine (lanes 1 and 3) and $[^{14}C]$glucosamine (lanes 2 and 4). $M_r$ values of major bands were calculated as in Fig. 1 and the positions of major glycosylated proteins are indicated.

**Table 1. Glycoprotein $M_r$ values of EHV-1 and EHV-4 calculated from Fig. 1 and Fig. 2**

<table>
<thead>
<tr>
<th>Terminology</th>
<th>EHV-1 structural glycoproteins</th>
<th>EHV-4 structural glycoproteins</th>
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<tr>
<td>gp300</td>
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<td>gp118</td>
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<td>gp108</td>
<td>108*</td>
<td>gp220</td>
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<td>gp45</td>
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* Denotes component of the disulphide-linked complex.

limiting dilution methods. Ascitic fluid containing monoclonal antibody was produced in BALB/c mice pre-sensitized with pristane.

**Immunoprecipitation.** Solubilized protein samples were incubated at room temperature with 2 $\mu$L of mouse ascitic fluid containing monoclonal antibody. After 1 h, 2 $\mu$L of rabbit anti-mouse IgG (Dako) was added, followed by 5 $\mu$L of washed formalin-fixed *Staphylococcus aureus* (Cowan strain) (Gibco). The incubation was mixed thoroughly.
Fig. 3. Analysis by diagonal gel electrophoresis of purified preparations of Abl and MD, either stained (a, b) or autoradiographs of $[^{14}C]$glucosamine-labelled (c, d) purified virus. Electrophoresis in the first dimension was from left to right under non-reducing conditions. $M_r$ values were calculated by running a marker lane of purified virus during the second dimension. The disulphide-linked glycoprotein subunits are indicated ($M_r \times 10^{-3}$) with arrows and the glycoproteins which have migrated slightly off the diagonal are marked with squares.
and held at room temperature for a further 1 h. The precipitate was then washed four times with RIPA buffer and immunoprecipitates were analysed by SDS-PAGE.

Western blotting. Transfer of protein from polyacrylamide gels to nitrocellulose sheets was carried out by the method of Towbin et al. (1979). Monoclonal antibody binding was detected by using sheep anti-mouse IgG-peroxidase conjugate (Dako) diluted to 1:1000. The peroxidase substrate was Hanker–Yates reagent and 0.01% H₂O₂.

RESULTS

Analysis of EHV-1 and EHV-4 structural proteins

Purification of extracellular EHV-1 and EHV-4 on either dextran, potassium tartrate or sucrose gradients yielded identical patterns of polypeptides resulting from SDS–PAGE analysis. The Coomassie Brilliant Blue-stained SDS–PAGE profiles of sucrose-purified EHV-1 (Ab1) and EHV-4 (MD) are shown in Fig. 1. The polypeptides of Ab1 are almost identical to those published for the high passage strain RAC-H (Killington et al., 1977), with no significant differences between the strains in Mr of the polypeptides. The profile obtained for strain MD although similar to that of Ab1 does have noticeable differences in the mobility of several polypeptides, particularly those in the Mr region of 92K to 60K. The [¹⁴C]glucosamine-labelled profiles are shown in Fig. 2 together with [³⁵S]methionine-labelled profiles. Both EHV-1 and EHV-4 have high Mr glycoproteins of >220K and apparently comparable glycoproteins: 118K/138K, 108K/112K, 88K/92K, 76K/74K, 60K/61K, 58K/59K and 45K/45K. This is in general agreement with the only other comparative analyses of EHV-1 and EHV-4 structural proteins (Turunen et al., 1981; Allen & Bryans, 1986). The high Mr glycoprotein of Ab1 appears as two distinct bands in some preparations of virus and also in infected cell extracts, whereas the profile shown in Fig. 2 has a single broad band which does not seem to be a doublet because of the intensity of the label. It is clear from comparisons on other gels that the 108K glycoprotein of Ab1 does not comigrate with the major structural protein of 112K labelled with [³⁵S]methionine and correspondingly the 112K glycoprotein of MD does not comigrate with the major structural protein of similar Mr. The Mr values of glycoproteins identified by analysis of data shown in Fig. 2 are displayed in Table 1 and they will be subsequently referred to by the prefix ‘gp’ followed by their Mr, as designated in Table 1.

Analysis of intramolecular disulphide bonds

The technique of two-dimensional gel electrophoresis under non-reducing/reducing conditions was chosen because it enabled clear identification of proteins linked together by disulphide bonds and the effect of reducing agents on the mobility of proteins in the presence of SDS. Proteins that do not contain intramolecular disulphide bonds migrate at an equal rate in both dimensions and thus will form a diagonal of spots migrating in direct proportion to their Mr value. Proteins containing intramolecular disulphide bonds will migrate slightly away from the diagonal due to non-stoichiometric binding of SDS under non-reducing conditions. Proteins containing intermolecular disulphide bonds will migrate as a high Mr form in the first dimension, and then break down to the lower Mr subunits on electrophoresis under reducing conditions.

Preparations of purified Ab1 and MD were solubilized in gel loading buffer without the reducing agent and samples were run on 15 cm tube gels. These were removed from the tube, soaked in buffer containing 10 mM-DTT, applied to the top of a slab gel and then run in the second dimension. Results are shown in Fig. 3 (a) and (b) for stained gels and Fig. 3 (c) and (d) for autoradiograms of [¹⁴C]glucosamine-labelled virus. Two polypeptides for both Ab1 and MD are visible on the stained gel which migrate below the diagonal. These correspond to the glycoproteins gp76 and gp58 for Ab1 and gp74 and gp61 for MD. The autoradiograms confirm this and establish that the bands are glycosylated. They also reveal that for Ab1 there is a distinct region of labelled material which remains above the diagonal and is likely to be high Mr complexes of the glycoprotein which have formed, because in this case the sample was not heated prior to electrophoresis in the second dimension (Snowden et al., 1985). There is also evidence of a polypeptide that remains on the diagonal directly above the disulphide-linked glycoproteins, which may represent a precursor. This is particularly evident for MD, as there is a poorly glycosylated species corresponding to about 95K directly below the band of gp220, and
Fig. 4. Immunoprecipitate of [14C]glucosamine-labelled detergent-solubilized antigen preparation of cells infected with EHV-1 strain Abl using antiserum prepared against the oligomeric form of HSV-1 gB. Lane 1 represents the infected cell extract and lane 2 the immunoprecipitate with the anti-oligomer serum. The \( M_r \) values of the precipitated proteins are shown on the right.

Fig. 5. Western blot analysis of the reaction of monoclonal antibody 9aC2 with purified preparations of Abl (lanes 1 and 2) and MD (lanes 3 and 4). Purified virus samples were either solubilized without sample heating (lanes 1 and 3) or with heating to 95 °C for 5 min (lanes 2 and 4). The 'oligomeric' forms of the glycoprotein detected for Abl are indicated with arrows. \( M_r \) values were determined by staining \( M_r \) markers run in a parallel lane transferred onto nitrocellulose. All four lanes were run on the same gel.

which is not present on the Abl gel. There are at least two proteins for both Abl and MD that migrated more rapidly in the absence of reducing agents than in their presence (indicated by squares), and this is likely to be due to intramolecular disulphide bonding.

**Interaction of polyclonal antiserum with the disulphide-linked glycoprotein**

Fig. 4 shows an autoradiogram of an SDS-PAGE profile of the immunoprecipitate of a [14C]glucosamine-labelled, detergent-solubilized, antigen preparation of EHV-1 strain Abl-infected RK13 cells using an HSV-1 gB antiserum (AO). This is a polyclonal rabbit antiserum prepared against the oligomeric form of HSV-1 gB (Snowden et al., 1985). Three glycosylated
species of $M_r$ 108K, 76K and 58K are precipitated from the EHV-1-infected cells by this serum. A similar antiserum from another rabbit has previously been shown to precipitate glycoproteins of $M_r$ 138K, 100K and 87K (Snowden et al., 1985) which, on reassessment have been allocated $M_r$ values of 140K, 108K and 76K respectively. Nothing was precipitated by the antiserum from similar extracts of uninfected cells. The HSV-1 polyclonal gB antisera have therefore been shown to precipitate EHV-1 glycoproteins of 140K, 108K, 76K and 58K.

**Interaction of monoclonal antibodies with the disulphide-linked glycoprotein**

A series of monoclonal antibody culture supernatants were screened by Western blotting using purified Abl and MD as the antigen source. Several culture supernatants reacted showing multiple bands and a typical profile of one (9xC2) is shown in Fig. 5. This antibody reacted strongly with Abl on the blot with two bands corresponding to 108K and 58K, and weakly with a diffuse area around 90K. Dilution of the antibody led to the gradual disappearance of the 90K region with continued reaction at 108K and 58K (data not shown). The antibody cross-reacted with MD but only with a single band on the blot, at 112K. If the Abl samples had not been heated prior to electrophoresis and subsequent blot transfer, the antibody reacted with a series of high $M_r$ regions as indicated by arrows in Fig. 5. These are probably due to protein aggregates and were only occasionally visible in unheated samples of MD.

Immunoprecipitation of $^{125}$I-labelled Abl and MD with 9xC2 led to the selective precipitation of a major 58K species for Abl and faint bands identified as 48K and 108K; a band of 112K was the major protein precipitated from $^{125}$I-labelled purified MD (Fig. 6). The identity of the minor bands is not known. These experiments, in conjunction with the results obtained from the diagonal gels, indicate that 9xC2 reacts with an epitope on gp58 of strain Abl which is also present on gp108, but not on gp76. It is likely, therefore, that this epitope is
conserved during proteolytic cleavage of gp108 of Ab1 but is destroyed during proteolysis of the equivalent gp112 polypeptide of MD.

A monoclonal antibody (αD3B4) was also identified which reacted with an Ab1 protein of Mr 140K on Western blots under non-reducing conditions and showed no reaction under reducing conditions (data not shown). Further investigations into the binding of this antibody by immunoprecipitation of [14C]glucosamine-labelled Ab1-infected cells (Fig. 7) revealed that when the sample was boiled but not reduced prior to electrophoresis a protein of Mr 140K was seen to have been precipitated. Reduction of the sample led to the appearance of three bands of Mr 108K, 76K and 58K with enrichment of the 76K band.

These data, in conjunction with those obtained with 9xC2 indicate that αD3B4 reacts with an epitope on gp76 or gp58 which is dependent on the conformation of the protein under non-reducing conditions and thus the apparent Mr of the mature protein is 140K. This cannot be applied to MD however, because αD3B4 does not cross-react with this virus.

**DISCUSSION**

The initial aims of these experiments were to enumerate and compare the major structural glycoproteins of EHV-1 and EHV-4 using purified virus preparations derived from recently isolated pathogenic strains of low passage history. The structural protein profiles, either stained with Coomassie Brilliant Blue R-250 (Fig. 1) or labelled with [35S]methionine (Fig. 2), show broad similarities with regard to the number of structural proteins and the range of Mr values. The [14C]glucosamine-labelled purified virus profiles are even more similar to each other, nine glycoproteins having been identified for EHV-1 and nine for EHV-4. For each virus at least three are related through precursor/product relationships. However, at this stage overall similarity of such profiles cannot be equated with functional equivalence of individual glycoproteins of comparable Mr values. The profiles are comparable to those of Turtinen et al. (1981) and Allen & Bryans (1986) but differences in the cell lines and polyacrylamide gels used make direct comparison very difficult at this stage.

When purified virus is run on two-dimensional gels with no reducing agent in the first dimension, it can be seen (Fig. 3) that the 76K and 58K glycoproteins of EHV-1 strain Ab1 and the 74K and 61K glycoproteins of EHV-4 strain MD are held together by disulphide bonds, a characteristic feature also shown by some other herpesviruses. Disulphide-linked glycoproteins of varicella-zoster virus (Grose et al., 1984; Keller et al., 1986), human cytomegalovirus (Farrar & Greenaway, 1986; Cranage et al., 1986), bovine herpesvirus type 1 (van Drunnen-Littel van den Hurk & Babiuk, 1986; Lawrence et al., 1986) and pseudorabies virus (Lukacs et al., 1985; Robbins et al., 1987) have all been shown to have extensive nucleic acid sequence homology with the HSV-1 gB gene with strong conservation of cysteine residues. These proteins also contain a region of basic amino acids (Lys/Arg) which is a typical proteolytic cleavage site present in many other glycoproteins that are modified by proteolysis (Bosch et al., 1981; Perez & Hunter, 1987; McCune et al., 1988). We believe, therefore, that the 76K and 58K glycoproteins of strain Ab1 and the 74K and 61K glycoproteins of strain MD are the gB homologues of EHV-1 and EHV-4 respectively. This belief is strengthened by the experiments on immunoprecipitation and Western blotting with polyclonal and monoclonal antisera. Snowden et al. (1985) and Snowden & Halliburton (1985) have previously shown that a minor glycoprotein of Mr 140K (previously 138K and reliably detectable only on labelling with 125I) is precipitated from EHV-1-infected cell extracts by a polyclonal rabbit antiserum to HSV-1 gB and that this polypeptide has a very similar tryptic peptide profile to HSV gB. Furthermore, they showed that the HSV-1 gB antiserum also reacts with a 108K and a 76K glycoprotein (formerly identified as 100K and 87K by analogy with the published data of Turtinen & Allen, 1982). Here we show that a similar polyclonal antiserum to HSV-1 gB immunoprecipitates a 140K, a 76K and a 58K glycoprotein from EHV-1-infected RK13 cells.

Monoclonal antibody 9xC2 reacts on Western blots or immunoprecipitates a 108K and a 58K glycoprotein from purified preparations of strain Ab1, and cross-reacts with a 112K glycoprotein of strain MD. Monoclonal antibody αD3B4 reacted on Western blots or immunoprecipitates a glycoprotein which under non-reducing conditions migrates with an
apparent Mr value of 140K with a trace of a 108K glycoprotein also present. Under reducing conditions the immunoprecipitated glycoproteins have Mr values of 76K and 58K with a trace of 108K also present. This latter monoclonal antibody does not cross-react with strain MD, but a recently isolated monoclonal antibody, 3A6B, reacts with strain MD in a manner identical to zD3B4 (data not shown). Taken together, the results of the two-dimensional gel electrophoresis, the Western blotting and the immunoprecipitations show that the EHV-1 gB homologue therefore migrates with an apparent Mr of 108K (140K under non-reducing conditions) but is cleaved to give glycoproteins of 76K and 58K which are normally held together by disulphide bonds. The EHV-4 gB homologue consists of a 112K glycoprotein which is cleaved to give glycoproteins of Mr 74K and 61K, also linked by disulphide bonds. Sequencing of one strand of the DNA of the gene of strain Abl that shares sequence homology with that of HSV-1 gB has confirmed the presence of a potential proteolytic cleavage site (W. A. Bonass, D. M. Elton, R. A. Killington, D. M. Meredith & I. W. Halliburton, unpublished results). The pattern of cross-reactivity of monoclonal antibody 9eC2 with EHV-1 and EHV-4 suggests that there may be differences in the location of the proteolytic cleavage site for the gB homologues. The epitope clearly exists in the uncleaved gp108 and gp112 of EHV-1 and EHV-4 respectively but whereas gp58 of EHV-1 also reacts with the antibody, proteolysis of the EHV-4 glycoprotein apparently destroys the epitope since 9eC2 reacts only with gp112 on Western blotting or immunoprecipitation. The failure of 9eC2 to coprecipitate gp76 of EHV-1 is at present not understood but similar monoclonal antibodies have been identified which react against components of the human cytomegalovirus gB homologue (Cranage et al., 1986; Gretch et al., 1988).

A recent publication (Allen & Yeargan, 1987) has reported the mapping of six major glycoprotein genes of EHV-1 using monoclonal antibodies and a Agtl1 expression system. One glycoprotein, termed gp14 and mapped to a location on the EHV-1 genome which is collinear with the HSV-1 gB gene, is, we believe, equivalent to the gp76 of the gB homologue. The monoclonal antibody used reacted only with one polypeptide by Western blotting and may therefore react with an epitope on this polypeptide which is not present in the uncleaved gp108 form. The other component of the gB homologue (gp58) seems to be equivalent to gp17/18 using their terminology and they map this glycoprotein to the U5 region of the EHV-1 genome. There are two possible explanations which may account for the apparent differences between the data presented here and those of Allen and Yeargan (1987). Firstly it is possible that purified preparations of the strain of EHV-1 they used contained no uncleaved form of the gB homologue which would account for the reaction with gp14 alone that they observed. Secondly, their monoclonal antibody to gp17/18 may not have reacted with the gp58 component of the gB homologue, but with another glycoprotein which comigrated with it. The profiles of purified EHV-1 published by Turtinen & Allen (1982) are very similar to those presented here for strain Abl and it is clear from their gels that there is a glycoprotein that migrates in a position corresponding to gp108; therefore we believe that their monoclonal antibody must react with an epitope which is present only on gp76. Careful observation of the diagonal gels reveals a glycoprotein which is of similar Mr to the gp58 component of the gB homologue. It is unclear at present whether or not this protein is related to the gB homologue but it is possible that this protein is encoded by the gene which maps to the U5 component of the EHV-1 genome.

Further experiments are now required to determine the relationship between the disulphide-linked gB homologues of EHV-1 and EHV-4. Purification of gp58 and gp76 has commenced and we intend to compare N-terminal sequence data obtained from those polypeptides with the amino acid sequence derived from the sequence of the EHV-1 gene which has homology with the HSV-1 gB gene.

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REFERENCES


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