Identification of the Envelope Surface Glycoproteins of Equine Herpesvirus Type 1

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SUMMARY

The structural polypeptides of purified enveloped virions of the Army 183 strain of equine herpesvirus type 1 (EHV-1) were examined by different analytical techniques to identify the envelope glycoproteins. Glycoproteins were identified by electrophoretic analysis in polyacrylamide slab gels of virus labelled \textit{in vivo} with $[^3\text{H}]$glucosamine or labelled enzymically \textit{in vitro} with either UDP-$[^{14}\text{C}]$galactose or sodium $[^3\text{H}]$borohydride. Fluorograms revealed eleven glycoproteins (mol. wt. 260000, 150000, 138000, 90000, 87000, 65000, 62000, 60000, 50000, 46000, and 24000). These glycoproteins probably correspond to virion protein (VP) 1-2, 9b, 10, 13, 14, 16, 17, 18, 21, 22a and 25 respectively, as designated in two other EHV-1 strains. In addition, a poorly resolved glucosamine-rich region (mol. wt. 250000 to 200000) corresponded to VP 3 to 8. The two isotopic surface labelling methods revealed that all the virus glycoproteins were exposed on the envelope surface.

The molecular anatomy of equine herpesvirus type 1 (EHV-1) has been most studied using the Kentucky A strain originally isolated from an aborted foetus and adapted to hamsters (KyA-ha) and to mouse L-M cells (KyA-LM) (Kemp \textit{et al.}, 1974; Perdue \textit{et al.}, 1974). The most recent definitive studies by Kemp \textit{et al.} (1974) identified 28 structural proteins of EHV-1 KyA-ha strains, ranging in mol. wt. from 270000 to 16000. Likewise, Perdue \textit{et al.} (1974) found a similar number and mol. wt. range of EHV-1 KyA-LM structural proteins, and reported that of these 28 structural proteins, nine were glycoproteins, four were lipoproteins and four were glycolipoproteins.

Because the published information on the glycoproteins of EHV-1 is limited additional analysis using techniques with increased resolving power are needed to better define the glycosylated proteins located on the surface of the EHV-1 envelope. Additionally, because of the importance of the role of herpesvirus glycoproteins in determining the host immune responses to virus infection, a more thorough identification and classification of the glycoproteins of EHV-1 are desirable.

Using a native EHV-1 strain, Army 183 (A183), passaged only in equines or equine cells, we identified glycoproteins of EHV-1 using a variety of analytical techniques including incorporation of radiolabelled carbohydrate precursors and enzymic addition of labelled carbohydrate groups.

Monolayer cultures of a diploid line of foetal dermis fibroblasts (KyED cells) were infected with the A183 strain of EHV-1 at an m.o.i. of 0-1 as previously described (Turtinen \textit{et al.}, 1981). After an adsorption period of 4 h at 35 °C, the inoculum was replaced with medium which contained either 4 $\mu$Ci/ml $[^3\text{H}]$glucosamine (60 Ci/mmol) or 1/10 the normal concentration of essential amino acids and 1 $\mu$Ci/ml of a $[^3\text{H}]$labelled amino acid mixture. The infection was allowed to proceed for approx. 35 h after which extracellular virus was purified by banding in preformed 28 to 40\% (w/v) potassium tartrate (KT) gradients in TE buffer (0.01 M-Tris-HCl, 0.001 M-EDTA, pH 7.4) to remove host membranes bound to the virion envelope as previously described in detail by Kemp \textit{et al.} (1974), Allen & Randall (1979) and Turtinen \textit{et al.} (1981). Polypeptides of purified EHV-1 were separated in 10\% polyacrylamide slab gels containing SDS as previously described by Turtinen \textit{et al.}(1981). Protein standards of known mol. wt. were included as reference markers. The standards included myosin (200000), $\beta$-galactosidase (135000), phosphorylase b (92000), bovine serum albumin (66000), ovalbumin (44000), carbonic anhydrase (30000), soybean trypsin inhibitor (22000) and lysozyme (15000). Proteins...
Fig. 1. Identification of EHV-1 A183 glycoproteins after labelling by various methods. Virus polypeptides were electrophoresed in a 10% polyacrylamide slab gel and subjected to fluorography to detect labelled polypeptides. The numbered $^3$H amino acid-labelled EHV-1 VPs in lane 1, and the EHV-1 VPs stained with Coomassie Brilliant Blue in lane 7 were used as reference markers for the labelled glycoproteins. Protein standards of known mol. wt. ($\times 10^{-3}$) are shown in lane 8. Glycoproteins in lane 2 were labelled with UDP-$^{14}$C galactose by the enzyme galactosyl transferase, in lane 4 with $^{3}$H glucosamine, and in lane 6 with sodium $^{3}$H borohydride after oxidation with galactose oxidase. Lanes 3 and 5 show longer film exposures of lanes 2 and 4 respectively.

were stained with Coomassie Brilliant Blue (CBB) as described previously by Allen & Randall (1979). Slab gels containing labelled polypeptides were prepared for fluorography by the procedure of Bonner & Laskey (1974). For detection of labelled polypeptides, Kodak XR-5 X-ray film was preflashed according to the method of Laskey & Mills (1975) and exposed to the dried gels for 1 to 14 days at $-80^\circ$C.

Numerical designation of the resolved EHV-1 polypeptides was based upon the nomenclature of O'Callaghan & Randall (1976) for the KyA-ha and KyA-LM strains (see Fig. 1). Previous comparison of the polypeptides of the A183 and KyA-ha strains of EHV-1 revealed very similar electrophoretic mobilities (Turtinen et al., 1981). The total number of well-resolved polypeptides was 22, and these ranged in mol. wt. from 260000 to 160000 (Fig. 1). Included among these structural polypeptides was a very high mol. wt. (> 250000) polypeptide apparently analogous to VP 1-2, and a very poorly resolved region between mol. wt. 250000 and 200000, corresponding to VP 3 to 8 of the standard nomenclature (O'Callaghan & Randall, 1976). Attempts to clearly resolve these high molecular weight proteins in 6% gels were unsuccessful (data not shown). It appears likely that this region may be mostly mucopolysaccharide containing glucosamine, as previously suggested by Killington et al. (1977).

Tritiated glucosamine was detected in at least nine proteins of EHV-1 (Fig. 1). These included VP 1-2, 3 to 8 region, 10, 13, 14, 17, 18, 21, and 25, ranging in mol. wt. from 260000 to 240000. Major peaks of $[^3]$H glucosamine radioactivity based on the intensity of the bands on the film corresponded to VP 13 (90000), VP 14 (87000), VP 1-2 (260000) and the VP 3 to 8 region (250000 to 200000). VP 10 (138000) and VP 18 (60000) contained lower amounts of radioactive label. A longer exposure of the labelled SDS–polyacrylamide gel bands to the film revealed a small amount of $[^3]$H glucosamine label in VP 17 (62000), VP 21 (50000) and VP 25 (24000).

KT gradient-purified virus was also labelled in vitro by two enzymic labelling methods specific
for exposed carbohydrate residues on the virion envelope surface. The enzymic addition of UDPM-[14C]galactose to the C-4 position in N-acetylglucosamine with galactosyl transferase was based on the procedure described by Wallenfels (1979). The reaction mixture contained (in a total volume of 460 µl) 0.25 M-Tris-HCl pH 7.4, 400 µg purified virus, 2 µCi UDP-[14C]galactose (270 mCi/mmol), 0.28 units galactosyl transferase and 0.02 M-MnCl2. Incubations were for 60 min at 37 °C on a shaker, followed by 5 min in an ice-bath. The labelled virions were washed to remove unreacted UDP-[14C]galactose by pelleting in TE buffer until no further label could be removed. At least eleven glycopolypeptides were resolved following SDS–polyacrylamide gel electrophoresis (SDS–PAGE) and fluorography (Fig. 1). The glycoproteins corresponded to VP 1-2, 3 to 8 region, 9b, 10, 13, 14, 17, 18, 21, 22a and 25, of either 3H amino acid-labelled or CBB-stained VPs run as reference markers.

Based on the intensity of the bands in the fluorogram, VP 13 and 14 contained the most label, followed by VP 21 and 22a. Two glycoproteins, VP 9b and 25, besides the VP 3 to 8 region, contained very little label and required longer exposure of the film for visualization of the labelled bands.

Virus glycoproteins were also labelled with sodium [3H]borohydride after oxidation of galactose groups with galactose oxidase (Gähmberg, 1978). In some cases, virions were pretreated with neuraminidase to remove terminal sialic acid residues in order to improve labelling (Luukkonen et al., 1977). The reaction mixture contained (in a total volume of 400 µl) 0.1 M-potassium phosphate buffer pH 7, 300 µg purified EHV-1 A183 virions, 36 units galactose oxidase and, in some experiments, 5 units neuraminidase. After 60 min at 37 °C, 10 µl (250 µCi, 282 Ci/mmol) sodium [3H]borohydride was added. The mixture was allowed to react for 30 min at room temperature. The virus was then pelleted by centrifugation and washed four times to remove free label.

Six surface glycoproteins of EHV-1 were labelled with the tritiated sodium borohydride procedure: these proteins included VP 1-2, 9b, 10, 14, 16 and 22a (Fig. 1). VP 22a was the most intensely labelled protein, followed by VP 1-2 and 14. VP 9b, 10 and 16 bound very little label with this technique. Although cleavage of terminal sialic acid with neuraminidase slightly improved the labelling of the virus, no additional proteins were labelled after neuraminidase treatment. No non-specific labelling occurred with tritiated sodium borohydride in the absence of galactose oxidase (data not shown).

All surface-labelled glycoproteins were solubilized to varying degrees by incubation with Nonidet P40 detergent at a concentration of 1-3% in TE buffer, followed by centrifugation at 100 000 g for 2 h (data not shown).

The results show at least twelve surface-exposed glycoproteins in the envelope of the A183 strain of EHV-1 (Table 1). Through the use of two in vitro labelling methods specific for different exposed carbohydrate moieties, three additional glycopolypeptides were detected which were not detected by in vivo labelling with [3H]glucosamine. VP 9b (150 000) and VP 22a (46 000) were detected by UDP-[14C]galactose labelling in which the [14C]galactose is enzymically attached to N-acetylglucosamine. VP 22a was also detected by labelling terminal galactose residues with the tritiated sodium borohydride/galactose oxidase method. The latter method was the only method of the three that found VP 16 (65 000) to contain carbohydrate. Eleven of the twelve glycoproteins were detected by at least two techniques.

Although only six EHV-1 glycoproteins were detected by the sodium borohydride/galactose oxidase method, in vitro labelling with UDP-[14C]galactose/galactosyl transferase exposed eleven surface glycoproteins indicating accessible N-acetylglucosamine moieties for labelling. This labelling technique correlated well with the in vivo labelling with [3H]glucosamine where nine of these eleven glycoproteins were detected (Table 1). Despite the lack of additional studies with other viruses, extensive studies by Wallenfels (1979) and Schindler et al. (1976) utilizing this method to label soluble glycoproteins or cell membranes indicate a high specific formation of a β-galactosidic bond on glycoproteins.

Nevertheless, only five of the twelve EHV-1 surface glycoproteins were detected by both in vitro surface labelling experiments. However, the failure to observe identical labelling with either method only indicates the limitations associated with any membrane labelling technique.
Table 1. Detection of EHV-1 virion structural glycoproteins (VP) by in vivo and in vitro labelling methods

<table>
<thead>
<tr>
<th>VP</th>
<th>Mol. wt. (× 10⁻³)</th>
<th>UDP-[¹⁴C]gal</th>
<th>[³H]glucosamine</th>
<th>[³H]borohydride</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-2†</td>
<td>260</td>
<td>+ ∨</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3-8§</td>
<td>200-250</td>
<td>+ ∨</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>9b</td>
<td>150</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>138</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>13</td>
<td>90</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>14</td>
<td>87</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>16</td>
<td>65</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>17</td>
<td>62</td>
<td>+</td>
<td>-</td>
<td>-</td>
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<tr>
<td>18</td>
<td>60</td>
<td>+</td>
<td>-</td>
<td>-</td>
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<tr>
<td>21</td>
<td>50</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>22a</td>
<td>46</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>25</td>
<td>24</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* Envelope surface-exposed glycoproteins of purified virions were labelled in vitro with UDP-[¹⁴C]galactose/galactosyl transferase or sodium [³H]borohydride/galactose oxidase as described in the text. Glycoproteins were labelled in vivo with [³H]glucosamine.

† VP 1-2 may consist of two polypeptides, but is resolved as one in 10% gels.

‡ +, Glycoprotein contained isotopic label detectable by SDS-PAGE and fluorography; –, glycoprotein not detectable.

§ The VP 3 to 8 region contained an unresolved number of polypeptides.

(Carraway, 1975). For example, most (if not all) surface glycoproteins contain galactose, but some galactose moieties may be inaccessible for labelling with galactose oxidase/tritiated sodium borohydride. Some galactose moieties on EHV-1 surface glycoproteins may be poorly exposed or subterminal and may not become labelled even after neuraminidase treatment. Such cryptic moieties occur on intact Semliki Forest virus where at least one envelope surface glycoprotein is more extensively labelled by this method if the virus envelope is disrupted (Luukkonen et al., 1977).

In the light of these findings for the A183 strain, continued identification of EHV-1 glycoproteins by more sensitive methods (e.g. two-dimensional gel electrophoresis) is needed to define poorly resolvable regions such as VP 3 to 8. Also a re-evaluation of the nomenclature for the glycoproteins of EHV-1 should be carried out to enable a more consistent description of any heterogeneity or function of glycoproteins among EHV-1 strains.

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Department of Veterinary Science  
University of Kentucky  
Lexington, Kentucky 40546, U.S.A.

LLOYD W. TURTINEN*  
GEORGE P. ALLEN

REFERENCES


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