Novel Herpes Simplex Virus Type 1 Glycoproteins Identified by Antiserum against a Synthetic Oligopeptide from the Predicted Product of Gene US4

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SUMMARY

Gene US4 of herpes simplex virus type 1 (HSV-1) has been predicted, from DNA sequence analysis, to encode a protein of molecular weight 25237 and its properties suggest it to be a membrane-associated protein. We have investigated this protein by raising antiserum to a synthetic oligopeptide corresponding to a stretch of amino acids from an internal hydrophilic region of the predicted sequence. This antiserum immunoprecipitates three glycoprotein species of apparent mol. wt. 37000, 48000 and 56000 from extracts of cells infected with HSV-1. These species are also specifically immunoprecipitated from purified virions. The in vitro translation product of gene US4 has an apparent mol. wt. of 35000. Sequence comparisons of the short unique regions of the HSV-1 and HSV-2 genomes, in combination with published mapping data for glycoprotein G (gG) of HSV-2, has led to the conclusion that the product of gene US4 of HSV-1 is the equivalent of gG.

Herpes simplex virus (HSV) produces several glycoproteins in infected cells. As constituents of the virion envelope and infected cell membrane these are important in many aspects of virus behaviour and host immune response. Four glycoprotein species, designated gB, gC, gD and gE, have been recognized for some time, and the HSV-1 and HSV-2 equivalents have been established by a variety of immunological, biochemical and genetic means (Eberle & Courtney, 1980; Halliburton, 1980; Hope et al., 1982; Lee et al., 1982; Marsden et al., 1978; Para et al., 1982, 1983; Pereira et al., 1980; Ruyechan et al., 1979; Zuzulak & Spear, 1983; Zweig et al., 1983). The DNA sequences for the genes encoding most of these have been determined (Bzik et al., 1984; Pellet et al., 1985; Frink et al., 1983; Draper et al., 1984; Dowbenko & Lasky, 1984; Swain et al., 1985; Watson et al., 1982; McGeoch et al., 1985). Two glycoproteins have been detected and characterized for which no equivalent proteins in the other serotype have yet been identified. One of these, gH, is encoded by HSV-1 and maps between coordinates 0.28 and 0.31 (Buckmaster et al., 1984). The other, g92K, maps in the short unique region of HSV-2 (Marsden et al., 1978, 1984) and is probably the same as the HSV-2 glycoprotein subsequently designated gG by Roizman et al. (1984). The original evidence for identity of g92K and gG, i.e. overlapping map positions, compatible molecular weights and no obvious equivalent HSV-1 glycoprotein, has recently been augmented by the observation that they share another property, that of being the only identified HSV-2 glycoprotein with affinity for Helix pomatia lectin (Olofsson et al., 1986). Based on this evidence we propose to redesignate g92K as gG in this and future publications.

Determination of the complete DNA sequence of the short unique region (Us) of HSV-1 has allowed prediction of the existence of as yet unidentified membrane-associated proteins (McGeoch et al., 1985; McGeoch, 1985). These contain recognized features of membrane-associated glycoproteins such as amino-terminal hydrophobic regions and potential transmembrane sequences. One such gene is US4 encoding a polypeptide of mol. wt. 25237. A hydrophobicity plot for the predicted product of HSV-1 US4 (Fig. 1) shows an amino-terminal...
hydrophobic region (1), a carboxy-terminal anchor sequence (3) and a possible transmembrane sequence (2). In this communication, we report the identification of previously unrecognized HSV-1 glycoproteins which react with an antiserum directed against a synthetic oligopeptide from part of the predicted protein sequence of the product of gene US4.

We examined the predicted US4 amino acid sequence, and chose an internal hydrophilic sequence of 11 amino acids, residues 115 to 125 (see Fig. 1). A dodecapeptide was synthesized corresponding to this with a C-terminal tyrosine to facilitate coupling to bovine serum albumin (BSA) (Bassiri et al., 1979). Rabbits were immunized with a conjugate of BSA and the peptide (Cambridge Research Biochemicals, Cambridge, U.K.). Anti-BSA antibodies were removed from the immune serum by absorption with BSA. Anti-peptide antibody was detected after 6 to 7 weeks by solid-phase radioimmunoassay and immunoprecipitation analysis of [3H]mannose-labelled extracts of BHK-21 clone 13 cells (Macpherson & Stoker, 1962) infected with HSV-1 strain 17 virus (Brown et al., 1973).

BHK cells were infected with HSV-1 (20 p.f.u./cell) and labelled with 100 μCi/ml [3H]mannose at 37°C in phosphate-buffered saline (PBS) from 5 to 11 h post-infection. From extracts of these, the anti-peptide serum (designated 13968) immunoprecipitated three glycoprotein species with apparent mol. wt. 37000, 48000 and 56000 (Fig. 2, lane 4). Specificity was demonstrated by pre-incubation of the antiserum with an excess of synthetic oligopeptide, thus blocking the specific interaction between antibody and protein (Fig. 2, lane 3).

To determine whether the polypeptides immunoprecipitated by antiserum 13968 were components of the virion, [35S]methionine-labelled HSV-1 virions were harvested from the medium of infected cells and purified on sucrose gradients (Stevely et al., 1985). Virion polypeptides are shown in Fig. 3, lane 1. Purified virions were sonicated, and incubated for 3 h at 4°C in extraction buffer containing 0.1 M-Tris–HCl pH 8, 10% glycerol, 0.5% NP40 and 0.5% sodium deoxycholate (Showalter et al., 1981). The extract obtained after clarification by centrifugation is shown in Fig. 3, lane 4. Immunoprecipitations with antiserum 13968 showed that the 37000 mol. wt. species, together with a heterogeneous species encompassing the 48000 and 56000 mol. wt. polypeptides, was present in HSV-1 virions (Fig. 3, lane 3). On 5 to 12.5% SDS–polyacrylamide gels the 56000 mol. wt. species migrated slightly faster than gD and the 48000 mol. wt. species faster than the precursor to gD. Antiserum 13968 immunoprecipitated the same polypeptides from an envelope/tegument fraction prepared by 5% NP40 extraction of purified virions (Fig. 3, lane 6). Although the background in these immunoprecipitations was high, probably due to the weak nature of the interaction between the anti-peptide antibodies and the protein, specificity was again demonstrated by pre-incubation of the immune serum with an excess of synthetic oligopeptide (Fig. 3, lanes 2 and 5). A large amount of gE was precipitated by
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Fig. 2. Immunoprecipitation of polypeptides from BHK cells infected with 20 p.f.u./cell HSV-1. Cells were infected or mock-infected at 37°C and labelled with [3H]mannose in PBS from 5 to 11 h post-infection. Extracts were made and immunoprecipitations carried out in extraction buffer as described previously (Frame et al., 1986) using Protein A-Sepharose to precipitate protein–antibody complexes. Immunoprecipitates were analysed on a 5 to 12.5% SDS-polyacrylamide gel. Lanes 1 and 2 show mock- and HSV-1-infected cell polypeptide profiles respectively. Lanes 3 and 4 show immunoprecipitations using infected cell extracts and the anti-oligopeptide serum (13968) respectively with and without prior incubation of the immune serum with 100 μg oligopeptide.

the anti-peptide serum, presumably as a result of the Fc binding capacity of gE. This has been observed with a number of rabbit anti-peptide sera and is variable from precipitation to precipitation.

In vitro translation experiments were carried out to identify the primary translation product of gene US4. Cytoplasmic RNA was prepared from BHK cells infected with 20 p.f.u./cell of HSV-1 (or mock-infected) for 6 h and translated in a fractionated rabbit reticulocyte system treated with micrococcal nuclease (Pelham & Jackson, 1976; Preston, 1979). Immunoprecipitations were carried out using the in vitro translated material and anti-peptide serum 13968. Fig. 4 (lane 4) shows that the primary translation product of gene US4 is a polypeptide of apparent mol. wt. 35000. Specificity was again confirmed by inhibition of this immunoprecipitation by pre-
incubation of the antiserum with an excess of synthetic oligopeptide (Fig. 4, lane 3). Lanes 1 and 2 show the $[^{35}S]$methionine-labelled polypeptide products of in vitro translated mock-infected and HSV-1-infected cytoplasmic RNA respectively.

The observation that the antiserum raised against a synthetic peptide from the predicted amino acid sequence of the glycoprotein product of gene US4 immunoprecipitates three different glycoproteins from extracts of HSV-1-infected cells and from purified virions could be explained in two ways. Firstly, they could be different forms of a single gene product, gene US4. Since all three species are components of HSV-1 virions (Fig. 3) this would be a unique situation for HSV. It is thought that the virion envelope may contain immature forms of glycoproteins.

Fig. 3. Immunoprecipitation of $[^{35}S]$methionine-labelled virion polypeptides. Lanes 1 and 4 show the polypeptide profiles of purified virions and a virion envelope/tegument preparation respectively. Lanes 2 and 3 show immunoprecipitation of virion polypeptides using antiserum 13968 with and without pre-incubation of the immune serum with 100 µg oligopeptide. Lanes 5 and 6 show immunoprecipitation of the envelope/tegument polypeptides with and without pre-incubation. The positions of known virion polypeptides are indicated on the left-hand side of the 5 to 12.5% SDS-polyacrylamide gel.

Fig. 4. Identification of the primary translation product of gene US4. Lanes 1 and 2 show the $[^{35}S]$methionine-labelled polypeptide products of mock- and HSV-1-infected cell mRNA respectively. Immunoprecipitations of the infected cell RNA translation products with antiserum 13968, with and without prior incubation with 100 µg oligopeptide, are shown in lanes 3 and 4 respectively.
**Table 1.** Approximate molar equivalents of 37 000, 48 000 and 56 000 proteins relative to known virion components

<table>
<thead>
<tr>
<th>Protein</th>
<th>Methionine residues per molecule</th>
<th>Mean area under peak from scan of purified virions (arbitrary units)*</th>
<th>Approximate molar quantity relative to gD</th>
</tr>
</thead>
<tbody>
<tr>
<td>gD</td>
<td>7 (McGeoch et al., 1985)</td>
<td>60012 ± 5353</td>
<td>1.00</td>
</tr>
<tr>
<td>gB</td>
<td>23 (Bzik et al., 1984)</td>
<td>136036 ± 2215</td>
<td>0.69 (+ 0.02)</td>
</tr>
<tr>
<td>gC</td>
<td>8 (Frink et al., 1983; Draper et al., 1984)</td>
<td>35887 ± 2067</td>
<td>0.52 (+ 0.05)</td>
</tr>
<tr>
<td>65K</td>
<td>16 (Dalrymple et al., 1985)</td>
<td>67507 ± 1603</td>
<td>0.49 (+ 0.02)</td>
</tr>
<tr>
<td>gE</td>
<td>9 (McGeoch et al., 1985)</td>
<td>24826 ± 1223</td>
<td>0.32 (+ 0.02)</td>
</tr>
<tr>
<td>37 000</td>
<td>3 (McGeoch et al., 1985)</td>
<td>6039 ± 438</td>
<td>0.24 (+ 0.02)</td>
</tr>
<tr>
<td>48 000</td>
<td>3 (McGeoch et al., 1985)</td>
<td>7678 ± 480</td>
<td>0.30 (+ 0.02)</td>
</tr>
<tr>
<td>56 000</td>
<td>3 (McGeoch et al., 1985)</td>
<td>8439 ± 926</td>
<td>0.33 (+ 0.04)</td>
</tr>
</tbody>
</table>

* Values represent mean and standard deviation of six ‘area under the curve’ estimations from a single densitometer scan.

when it is formed at the nuclear membrane, but that these are processed to the mature forms as the virions are transported to the cell surface (Spear, 1984). This is certainly clear for glycoproteins B and D where only the final forms can be detected in virions extruded from infected cells (Johnson & Spear, 1982). The second possibility is that the three glycoproteins may not all be products of gene US4. In this case any one of these could be the expressed product of US4 and the others complexed to it. There is, however, no documented evidence for the existence of complexes between heterologous HSV glycoproteins.

Therefore, the final form of the glycoprotein product of gene US4 of HSV-1 has not yet been unambiguously identified. Although the *in vitro* translation product of this gene is clearly a polypeptide of 35 000 mol. wt., the anti-oligopeptide serum against part of the amino acid sequence of the predicted gene product immunoprecipitated the three glycoproteins (apparent mol. wt. 37 000, 48 000 and 56 000) described above. To our knowledge these glycoproteins have not previously been recognized in HSV-1-infected cells or in purified virions.

We have estimated the quantity of these glycoproteins in the virion in terms of molar equivalents relative to gB, gC, gD, gE and the 65 000 mol. wt. (65K) virion protein responsible for activation of immediate early gene transcription (Batterson & Roizman, 1983; Campbell et al., 1984; Dalrymple et al., 1985). A densitometer scan of [35S]methionine-labelled purified virion polypeptides was carried out and the areas under the peaks for each of these was determined (Table 1). After normalization for methionine content the amounts of the 37 000, 48 000 and 56 000 mol. wt. glycoproteins were found to be approximately half that of gC and 65K, slightly less than half that of gB and about one-third that of gD (Table 1). In this analysis one assumption was that all three species were products of gene US4 and therefore contained three methionine residues (excluding the signal peptide). As discussed above, this assumption may not be justified. Another assumption was that the peaks evaluated represent a single protein species. Again this may not be the case for all the proteins analysed. Although this approach is subject to error our estimates suggest that the glycoprotein product of gene US4 of HSV-1 is not a minor species and its previous lack of detection is more likely due to its relatively low molar methionine content.

Finally, sequence analysis of the U₅ region of HSV-2 DNA has shown that HSV-2 possesses a gene which is partially homologous to HSV-1 US4 and is thought to encode a glycoprotein, but which is considerably larger than HSV-1 US4 (D. J. McGeoch, D. McNab & H. W. M. Rixon, unpublished results; McGeoch, 1985). This is the region of the genome in which gG-2 maps (Marsden et al., 1978, 1984; Roizman et al., 1984; Olofsson et al., 1986) and since there are no other potential large glycoprotein genes in HSV-2 U₅ to the left of the gD gene, as judged by DNA sequence interpretation, it follows that the HSV-2 equivalent US4 protein is gG. The HSV-1 US4 protein is therefore the HSV-1 equivalent of gG-2.
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REFERENCES


LEE, G. T.-Y., PARA, M. F. & SPEAR, P. G. (1982). Location of the structural genes for glycoproteins gD and gE and for other polypeptides in the S component of herpes simplex type 1 DNA. Journal of Virology 43, 41-49.


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