Characterization of a Herpes Simplex Virus Type 2-specified Glycoprotein with Affinity for N-Acetylgalactosamine-specific Lectins and Its Identification as g92K or gG

By SIGVARD OLOFSSON, * MARITA LUNDSTRÖM, HOWARD MARSDEN, 1 STIG JEANSSON AND ANDERS VAHLNE

Department of Virology, Institute of Medical Microbiology, University of Göteborg, Guldhedsrgatan 10 B, S-413 46 Göteborg, Sweden and 1 Institute of Virology, University of Glasgow, Church Street, Glasgow G11 5JR, U.K.

(Accepted 31 December 1985)

SUMMARY

Extracts from herpes simplex virus type 2 (HSV-2)-infected cells were subjected to affinity chromatography with gel-bound Helix pomatia lectin (HPA). Only one HSV-2-specified glycoprotein was isolated by this procedure and the glycoprotein had an apparent molecular weight of 130 000 (130K). The HPA-binding glycoprotein was genetically mapped, using HSV-1 × HSV-2 intertypic recombinants into the short component of the HSV-2 genome. The mapping position, electrophoretic mobility and the antigenic properties of the HPA-binding protein indicated that it was unrelated to glycoprotein C (gC), which is the HPA-binding glycoprotein in HSV-1-infected cells, and distinct from gE and gD which map in the S component. The glycoprotein was almost quantitatively precipitated by monoclonal antibody AP1, specific for glycoprotein g92K and it also reacted with monoclonal antibody 1206-3, specific for the HSV-2 glycoprotein G previously described. It is concluded that the isolated glycoprotein is identical to g92K and consequently also to the HSV-2-specific glycoprotein G.

INTRODUCTION

Like most other membrane glycoproteins of both viral and cellular origin, herpes simplex virus type 1 (HSV-1) glycoproteins contain N-glycosyl oligosaccharides (Hope & Marsden, 1983; Pizer et al., 1980; Serafini-Cessi & Campadelli-Fiume, 1981; Wenske et al., 1982). In addition to these carbohydrates, one of the HSV-1-specified glycoproteins, designated gC-1, also contains O-glycosyl oligosaccharides with affinity for the N-acetylgalactosamine (GalNAc)-binding Helix pomatia lectin (HPA) (Olofsson et al., 1981, 1983a). This lectin has been reported to bind to a restricted number of surface carbohydrates only, including biologically active structures such as blood group A substance, the Forssman antigen and some differentiation antigens glycoprotein in nature (Gahmberg & Andersson, 1982; Goldstein & Hayes, 1978; Hammarström et al., 1973). Although other HSV-1 glycoproteins contain O-linked oligosaccharides (Johnson & Spear, 1983), the HPA-binding activity is associated largely, if not exclusively, with gC-1 (Olofsson et al., 1981, 1983b). The HPA-binding activity appears thus to be a factor distinguishing gC-1 from other HSV glycoproteins.

Recently, a glycoprotein with similar exclusive affinity for HPA was reported also for HSV type 2 (HSV-2)-infected cells (Svennerholm et al., 1984; Suchanova et al., 1984). The aim of the present paper is to characterize the type 2 HPA-binding glycoprotein and to determine its relationship to gC-1.

METHODS

Viruses and cells. HSV-1 strain F was obtained from Dr B. Roizman. HSV-2 strain B 4327 UR was characterized with monoclonal antibodies as described by Nilheden et al. (1983). An established line of green monkey kidney
cells (GMK AH-1) was used throughout the study. Eagle's MEM supplemented with 10% newborn calf serum and antibiotics was used for propagation of cells while the same medium without serum was used for maintenance of the cultures. In the mapping studies intertypic recombinants between HSV-1 (strain 17 syn *) and HSV-2 (HG52), isolated and characterized previously (Preston et al., 1978; Marsden et al., 1978, 1982, 1984; Chartrand et al., 1981; Davison et al., 1981; Hope et al., 1982), were used.

Chemicals, radiochemicals and chromatographic media. D-[6-3H]Glucosamine (30 Ci/mmol) was purchased from Amersham and Helix pomatia lectin-Sepharose 6MB and soybean lectin (SBA) were obtained from Pharmacia. SBA was coupled to Affi-Gel 10 (Bio-Rad) as previously described (Olofsson et al., 1981).

Detergent extraction of infected cells. GMK cells in 50 mm Petri dish cultures were infected with HSV-1 at a m.o.i. of 10 to 20. After 1 h of adsorption, the inoculum was replaced by maintenance medium, and at 4 h post-infection the cultures were radiolabelled with 100 μCi [3H]glucosamine in 1 ml of the same medium. The cells were harvested and extracted with 1% Triton X-100 in Tris-buffered saline (TBS: 0.15 M-NaCl, 0.02 M-Tris-HCl pH 7.5) at 16 h post-infection as previously described (Olofsson et al., 1981). A soluble Triton X-100 extract resulting from centrifugation at 100000 g for 1 h was used for further experiments.

Lectin affinity chromatography. A micromethod for lectin affinity chromatography was used. Two-hundred μl of glycoproteins or digests were applied to 1 ml of gel-bound lectin in 6 mm-wide columns (Olofsson et al., 1981). The materials were allowed to adsorb for 15 min at room temperature (HPA) or +4 °C (SBA). After adsorption, the gels were washed with 10 bed volumes of Triton X-100 in TBS and subsequently eluted with 5 mM-GalNAc in the same buffer. The temperature conditions mentioned above were maintained throughout the experiment.

Antibodies. Monoclonal antibodies were prepared and concentrated as previously described (Olofsson et al., 1983a). The monoclonal antibody against gC-1 (designated B1C1) has been characterized previously (Olofsson et al., 1983a) and the monoclonal antibody designated B3GI1 was specific for gB-1 as demonstrated by immunoprecipitation with [3H]glucosamine- and [3H]leucine-labelled infected cell extracts (S. Jeansson et al., unpublished observations). A third monoclonal antibody (designated O1C5) was prepared from mice immunized with the eluted fraction from a HPA chromatography of extracts from HSV-2-infected cells. A monoclonal antibody (AP1) specific for g92K (Marsden et al., 1982) was supplied by Dr A. C Minson, University of Cambridge, U. K. and the gG-2-specific monoclonal antibody 1206-3 was supplied by Dr L. Pereira, California Department of Public Health, U.S.A. Also, the polyclonal monospecific rabbit antibody against gC-1 (designated K642) has been characterized previously (Olofsson et al., 1983a). Finally, a rabbit hyperimmune serum (K70) was prepared by infection with purified HSV-2 (Jeansson, 1972).

Radioimmunoprecipitation and SDS-polyacrylamide gel electrophoresis (SDS-PAGE). Monoclonal antibodies monospecific for HSV glycoproteins, in volumes of 100 μl culture supernatants, were allowed to react with 100 μl [3H]-labelled glycoprotein preparations under gentle agitation for 1 h at 4 °C. One-hundred μl of a 10% (w/w) suspension of formalin-fixed Staphylococcus aureus (strain Cowan I), coated with rabbit anti-mouse IgG antibody (Dako, Copenhagen, Denmark), were added and the mixture was subjected to gentle agitation at +4 °C for 1 h. Alternately, a polyclonal anti-gC monospecific rabbit serum, prepared as described previously (Olofsson et al., 1983a), was used but allowed to react with uncoated bacteria. The bacteria were washed twice in TBS containing 1% Triton X-100 and 0.1% bovine serum albumin and twice in the same buffer without albumin. The final pellets were solubilized and subjected to SDS-PAGE as described by Morse et al. (1978) in a separation gel of 9-25% (w/w) acrylamide, crosslinked with 0.25% diallyltartardiamide. The gels were treated with ‘Enlightening’ (New England Nuclear) and subjected to fluorography on Kodak X-Omat AR film. 14C-methylated proteins (14K to 200K, Amersham) were used as molecular weight standards.

Mapping of HSV-2-specific HPA-binding glycoprotein. Detergent extracts from [3H]glucosamine-labelled cells infected with HSV-1 × HSV-2 recombinants were prepared as described above. Monoclonal antibodies B1C1 or O1C5 in volumes of 100 μl were mixed with radiolabelled extracts (100 μl) and processed as described above for the materials subjected to SDS-PAGE. After four washes the staphylococcal material was boiled in 5% SDS and counted in a Beckman LS 2800 scintillation counter calibrated for d.p.m. determinations.

RESULTS

Isolation of an HPA-binding HSV-2-specific glycoprotein

A detergent extract from [3H]glucosamine-labelled HSV-2-infected cells was subjected to HPA affinity chromatography. About 10% of the radiolabelled material bound specifically to the lectin (Fig. 1). SDS-PAGE of the eluted fractions demonstrated that the HPA-binding activity was associated with one single polypeptide band with an apparent mol. wt. of about 130K (Fig. 2g). It was also found that SBA, another GalNAc-specific lectin, bound a glycoprotein of the same apparent molecular weight (Fig. 2f). It was concluded that the HPA-binding glycoprotein was induced by HSV because it could not be detected in uninfected cells and it was precipitable by an HSV-2 antiserum obtained after infection of a rabbit with purified
Characterization of the HPA-binding HSV-2-specific glycoprotein

As the HPA-binding activity of HSV-1-infected cells is associated mainly, if not exclusively, with gC-1 (Olofsson et al., 1981, 1983b) it seemed likely that the HPA-binding HSV-2-specified glycoprotein could be related to gC-2. To identify gC-2 we used a rabbit antiserum (K642) obtained after immunization with gC-1, purified by affinity chromatography with gC-1-specific monoclonal antibody (Olofsson et al., 1983a). This antibody precipitated two HSV-2 polypeptides migrating in the 70K to 80K region (Fig. 2e).

Owing to their molecular weight and reactivity with a polyclonal, exclusively gC-specific antiserum, we concluded that these polypeptides were related to or identical with gC-2, recently characterized and defined by Zezulak & Spear (1983) and Zweig et al. (1983). No HSV-2-specified glycoprotein in the 130K region was precipitated by antiserum K642 (Fig. 2e). It was also found that this antiserum precipitated less than 1% of the eluted fraction from the HPA-binding fraction of [3H]glucosamine-labelled extracts from HSV-2-infected cells. In conclusion, the data indicated that the HPA-binding glycoprotein was distinct from gC-2.

Genetic mapping of the HSV-2-specified HPA-binding glycoprotein

An HSV-2 type-specific monoclonal antibody prepared from mice immunized with purified HPA-binding glycoprotein was used as a probe in the mapping studies. This monoclonal antibody (designated O1C5) precipitated one single [3H]glucosamine-labelled polypeptide with a slightly higher apparent molecular weight (about 130K) than the HPA-binding glycoprotein fraction from HSV-2-infected cells (Fig. 3). A similar phenomenon has been observed for gC-1,
Fig. 3. Electrophoretic characterization of the glycoprotein precipitated by monoclonal antibody O1C5. (a) Radioimmunoprecipitation with monoclonal antibody O1C5 of a total [3H]glucosamine-labelled extract from HSV-2-infected cells. (b) HPA-binding fraction from the same extract (see Fig. 1). Positions of 14C-labelled molecular weight marker peptides are indicated.

Fig. 4. Summary of the genomic structures of the 12 recombinants used in this report. The genome arrangement of HSV DNA is illustrated at the top of the figure showing the long and short repeat sequences and the long and short unique regions. Those sequences of the recombinant derived from the type 1 and type 2 parent are represented by a thick continuous line superimposed on the upper (HSV-1) and lower (HSV-2) of the two horizontal dotted lines. Crossover regions are indicated by one or two vertical lines between the thick continuous horizontal lines.

in as much as the HPA-binding fraction of gC-1 has a higher mobility that the non-binding fraction probably caused by the differences in carbohydrate composition (Olofsson et al., 1983b). However, more than 85% of the eluted HPA-binding fractions from the chromatography shown in Fig. 1 was precipitated by this antibody, confirming that the HPA-binding glycoprotein was recognized by the antibody.

In the mapping study, [3H]glucosamine-labelled extracts from GMK cells infected with the intertypic recombinants were subjected to radioimmunoprecipitation with O1C5. The genomic structures of these recombinants are shown in Fig. 4. The amount of radioactivity precipitated is shown in Table 1, expressed as a percentage of the total radiolabel added to the reaction mixture. Control experiments with extracts from uninfected cells showed that the background was 0.7 ± 0.1% (M ± s.e.m., n = 5). All results less than 1.5% (2 × background) were scored as negative. Using this background limit, clearcut results were obtained with all recombinants, indicating a mapping position delimited on the left by recombinant RD104 and on the right by R12-1 (0.892
Characterization of gG-2

Table 1. Mapping of HSV-2-specified HPA-binding glycoprotein by radioimmunoprecipitation with monoclonal antibody*

<table>
<thead>
<tr>
<th>Virus</th>
<th>Total activity (d.p.m. × 10⁻⁶)</th>
<th>D.p.m. precipitated (% of total)</th>
</tr>
</thead>
<tbody>
<tr>
<td>17 syn⁺ (HSV-1)</td>
<td>0.4</td>
<td>0.6</td>
</tr>
<tr>
<td>HG52 (HSV-2)</td>
<td>0.6</td>
<td>27.3</td>
</tr>
<tr>
<td>Bx6(17-1)</td>
<td>0.6</td>
<td>5.0</td>
</tr>
<tr>
<td>Bx1(28-1-1)</td>
<td>0.6</td>
<td>25.1</td>
</tr>
<tr>
<td>RD104</td>
<td>0.7</td>
<td>0.8</td>
</tr>
<tr>
<td>RD113</td>
<td>1.3</td>
<td>0.4</td>
</tr>
<tr>
<td>RD213</td>
<td>1.5</td>
<td>0.4</td>
</tr>
<tr>
<td>RE 4</td>
<td>0.9</td>
<td>0.3</td>
</tr>
<tr>
<td>RE 6</td>
<td>0.6</td>
<td>0.8</td>
</tr>
<tr>
<td>R12-1</td>
<td>0.7</td>
<td>0.9</td>
</tr>
<tr>
<td>17×x11'(1)</td>
<td>0.4</td>
<td>24.0</td>
</tr>
<tr>
<td>Bx1(24)</td>
<td>1.1</td>
<td>22.8</td>
</tr>
<tr>
<td>RH6</td>
<td>0.6</td>
<td>0.9</td>
</tr>
<tr>
<td>RD216</td>
<td>0.7</td>
<td>0.6</td>
</tr>
</tbody>
</table>

*[^3H]Glucosamine-labelled extracts from GMK cells infected with the intertypic recombinants indicated were subjected to radioimmunoprecipitation with monoclonal antibodies against HSV-2-specified HPA-binding glycoprotein (O1C5).

Finally, the HPA-binding glycoprotein from HSV-2-infected cells was subjected to radioimmunoprecipitation with monoclonal antibody AP1, which was used as a probe for g92K by Marsden et al. (1984) and monoclonal antibody 1206-3 used for characterization of gG-2 by Roizman et al. (1984). Both antibodies precipitated 85% of the HPA-binding HSV-2-specified glycoprotein. When tested in the ELISA system, described by Svennerholm et al. (1984) both monoclonal antibodies demonstrated titres higher than 100,000 against the HPA-binding glycoprotein from HSV-2-infected cells and less than 50 against the corresponding antigen from HSV-1-infected cells. The mapping data and the similar antigenic reactivity therefore indicated that the HPA-binding HSV-2-specified glycoprotein was identical with g92K and gG-2.

DISCUSSION

This paper shows that only one of the HSV-2-specified glycoproteins has affinity for the GalNAc-binding lectins HPA and SBA. Similar lectin affinities were reported previously for gC but for no other HSV-1-specified glycoprotein (Olofsson et al., 1981, 1983b). Judging from these unique lectin-binding properties it seemed reasonable to assume that the HSV-2-specified HPA-binding glycoprotein was related to or identical with gC-2. However, our data from the radioimmunoprecipitation analysis and subsequent SDS-PAGE were not compatible with this assumption. Furthermore, the mapping position of the HPA-binding HSV-2-specified glycoprotein (as discussed in some detail below) was found to be in the S component, situated at least 0.2 map units from the map position of gC-2, delimited by 0.62 and 0.64 map units (Zeuzulak & Spear, 1984). In conclusion, both immunological and mapping data indicate that gC-2 and the HPA-binding glycoproteins are two distinct HSV-2-specified glycoprotein species.

The mapping studies in the present report were performed with the same HSV-1 × HSV-2 intertypic recombinants as used by Marsden et al. (1984) for the characterization of a HSV-2-specific glycoprotein, designated g92K. Previous studies using 18 HSV-1 × HSV-2 intertypic recombinants concordantly gave a genomic location for g92K between coordinates 0.846 and 0.924 (Marsden et al., 1978, 1984). However, two of them R12-3 and RD104 gave mutually incompatible locations. More recently, the genome structures of a number of the recombinants have been investigated in more detail. These studies (A. C. Minson, personal communication) revealed that the stock of R12-3 appeared to be a mixture of two populations and contained previously undetected HSV-2 sequences in Us. Consequently, R12-3 cannot be used for
mapping purposes. The effect of omitting the data for R12-3 on our previously published map position for g92K is to narrow the location further, to between coordinates 0.892 and 0.924, delimited on the left by RD104 at the HSV-2 BglII q-1 site and on the right by R12-I at the HSV-2 BamHI c'-d' site. The results indicated similar mapping positions for g92K and the HPA-binding glycoprotein in the S component delimited by map coordinates 0.892 and 0.924 (Marsden et al., 1984). Of particular significance was the observation that recombinant R12-1 did not induce the HPA-binding HSV-2 glycoprotein (Table 1) since this recombinant has been shown (Marsden et al., 1984) to induce gE-2 and a gD with mobility distinct from that induced by HSV-1 or HSV-2. Taken together, these results suggest that like g92K the HPA-binding HSV-2 glycoprotein is encoded by a region in the S component at least in part different from that coding for gD and gE. In conclusion, the mapping data and the finding that the HPA-binding glycoprotein was almost quantitatively precipitated by the g92K-specific monoclonal antibody AP1 indicate that g92K and the HPA-binding HSV-2-specified glycoprotein are identical. The different apparent molecular weight (130K) reported in this paper is due to different electrophoretic systems used in the present study and in that of Marsden et al. (1984).

Recently, an HSV-2-specified glycoprotein, designated gG, was identified as a fully glycosylated polypeptide (i) with apparent mol. wt. of 124K to 136K, (ii) mapping in the S component and (iii) reacting with an HSV-2-specific monoclonal antibody, designated H 966 or 1206-3 (Roizman et al., 1984). The HPA-binding HSV-2-specified glycoprotein fulfils all these criteria. Further similarities between gG-2 and the HPA-binding glycoprotein is a dominant type-specific immunological reactivity (Roizman et al., 1984; Svennerholm et al., 1984) and that no HSV-1-specified counterpart mapping in the same region has been identified. Consequently, our data indicate that the HPA-binding glycoprotein in the present study and gG-2 in fact are one and the same glycoprotein.

Affinity for GalNAc-specific lectins is a phenotypic property of gC-1 and gG-2 but no other HSV glycoproteins including gC-2. One intriguing question is whether or not this property also reflects similarities in the amino acid sequences between these two glycoproteins. In other words, which is the mechanism selecting only these two glycoproteins for addition of HPA-binding carbohydrates? One of three explanations seems plausible. (i) The genes encoding gC-1 and gG-2 belong to the same transcriptional class, permitting peak biosynthesis of both glycoproteins at a selected period of time when the necessary glycosyl transferases are active. (ii) Both glycoproteins contain signals for transport to special compartments of the cells where the necessary glycosyl transferases are expressed. The existence of such peptide signals directing viral glycoproteins to subcellular locations has been postulated for other viruses (Rodriguez-Boulan & Sabatini, 1978; Morrison & Ward, 1984). (iii) The addition of HPA-binding oligosaccharides requires special attachment sequences only present in gC-1 and gG-2. The peptide signal requirements for addition of N-glycosyl oligosaccharides are known in detail (Ronin et al., 1978), but very little is known about such structural factors governing the attachment of O-glycosyl oligosaccharides (Kessler et al., 1979; Takayashu et al., 1982). As HSV glycoproteins other than gC-1 and gG-2 contain O-linked carbohydrates (Johnson & Spear, 1983), this explanation would imply that the peptide structure also might influence terminal properties of O-linked oligosaccharides.

Explanations (ii) and (iii) strongly implicate similarities in at least some part of the amino acid sequences of gC-1 and gG-2. Such similarities have not been detected immunologically (Zezulak & Spear, 1983; Roizman et al., 1984; Marsden et al., 1984) but they may be masked by the large HPA-binding carbohydrates (Olofsson et al., 1983a). Any special peptide sequence in gC-1 responsible for the addition of HPA-binding oligosaccharides should be absent or aberrant in gC-2. It is therefore interesting that although there is a high degree of homology between the amino acid sequences of gC-1 and gC-2 it was shown by two laboratories that gC-1 contains a 27 amino acid sequence, near the amino terminus, which appears to be totally lacking in the gC-2 sequence (Dowbenko & Lasky, 1984; Swain et al., 1985). It was suggested that there is a more pronounced evolutionary divergence between gC-1 and gC-2 than between other HSV-1 glycoproteins such as gB-1, gD-1 and gE-1, and their HSV-2-specified counterparts (Zezulak & Spear, 1983). The results of the present paper seem to be another example of this divergence.
The gG-2-specific monoclonal antibody was a kind gift from Dr L. Pereira. We wish to thank Dr A. C. Minson for the results of his investigation of recombinant R12-3. The skilful technical assistance of I. Sjöblom and E. Sjögren-Jansson is gratefully acknowledged. This work was sponsored by the Swedish Medical Research Council (Grant No. 4514) and the Medical Faculty of the University of Göteborg.

REFERENCES


JEANSSON, S. (1972). Differentiation between herpes simplex virus type 1 and type 2 strains by immunoelectrophoresis. Applied Microbiology 24, 96-100.


(Received 7 October 1985)