Antigenic and Biochemical Analysis of gB of Herpes Simplex Virus Type 1 and Type 2 and of Cross-reacting Glycoproteins Induced by Bovine Mammillitis Virus and Equine Herpesvirus Type 1

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(Accepted 15 October 1984)

SUMMARY

An antiserum was produced to the oligomeric form of glycoprotein B (gB) induced by herpes simplex virus type 1 (HSV-1) strain 17. This antiserum gave a single common precipitin line in agar gel immunodiffusion with HSV-1, HSV-2, bovine mamillitis virus (BMV) and equine herpesvirus type 1 (EHV-1). It also neutralized HSV-1, HSV-2 and BMV but not EHV-1. Absorption of the antiserum with excess HSV-2 or BMV antigen resulted in an HSV-1-specific neutralizing antiserum. In immunoprecipitation, two proteins, gB and pgB, were precipitated from HSV-1- and HSV-2-infected cells and at least three from BMV- and EHV-1-infected cells. Glycoprotein B and pgB of three HSV-1 and three HSV-2 strains and the corresponding antigenically related glycoproteins of BMV- and EHV-1-infected cells were labelled with $^{125}$I, digested with trypsin and the resulting peptides separated by two-dimensional thin-layer chromatography or high-pressure liquid chromatography. The resulting profiles were found to be almost identical, suggesting considerable structural conservation of the peptide backbone of the antigenically related glycoproteins of these four viruses.

INTRODUCTION

Four herpes simplex virus (HSV)-specific glycoproteins designated gB, gC, gD and gE (Spear, 1976; Baucke & Spear, 1979) plus several other uncharacterized minor glycoproteins have been detected on the virion envelope and in the plasma membrane of HSV-infected cells (Sârmiento et al., 1979; Para et al., 1980; Balachandran et al., 1981; Peake et al., 1982; Marsden et al., 1984). Because of their surface position, it seems certain that they are involved with the immune system, including acting as targets for virus-neutralizing antibody (Powell et al., 1974; Cohen et al., 1978; Eberle & Courtney, 1980a). Antiserum to HSV-1 contains both specific and cross-reacting neutralizing antibodies (Sim & Watson, 1973). The latter react with several herpesviruses including HSV-2, bovine mamillitis virus (BMV), herpesvirus B, herpesvirus tamarinus and, possibly, canine herpesvirus (Watson et al., 1967; Hampar et al., 1969; Blue & Plummer, 1973; Aurelian, 1968). The identification of which glycoprotein induces which particular type of antibody is being investigated by purification of individual glycoproteins for the production of monoprecipitin antiserum (Eberle & Courtney, 1980a) and by the production of monoclonal antibodies to HSV glycoproteins. From such studies it is now certain that gD induces both type-specific and cross-reacting neutralizing antibodies (Watson & Wildy, 1969; Sim & Watson, 1973; Cohen et al., 1978) some of which require complement to neutralize (Eisenberg et al., 1982). Anti-gD serum also neutralizes BMV (Killington et al., 1978). However, the role of gB and gC in cross-reactions with other viruses is not certain. So far, it has been demonstrated that gB and gC may induce type-specific neutralizing antibodies (Powell et al.,

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1974; Eberle & Courtney, 1980a) and cross-reactive sites are present on gB not only with HSV-1 and HSV-2, but also with BMV and B virus (Norrild et al., 1978).

The mobility of glycoproteins on SDS–PAGE has been shown to be variable between strains of a particular serotyple and also to vary with the same strain grown in different cell lines (Halliburton, 1980). Furthermore, several intertypic recombinants produced from mixed infections of HSV-1 and HSV-2 produce glycoproteins of mobility characteristic of neither parent (Halliburton, 1980). Finally, variation in antigenic sites involved in neutralization of HSV-1 strains has been reported (Killington et al., 1978).

These findings have led us to examine the structural properties of herpesvirus-induced glycoproteins in relation to their possible cross-reactivities and sources of variation. This paper reports studies on gB. We have produced an antiserum to the oligomeric form of gB (Sarmiento & Spear, 1979), characterized it by agar gel immunodiffusion, neutralization and immune precipitation and compared the polypeptides immunoprecipitated by this antiserum from cells infected with four different herpesviruses using tryptic peptide analysis.

**METHODS**

**Cells and media.** BHK 21 and RK 13 cells were grown as described by Watson et al. (1966) in the autoclavable Glasgow modification of Eagle’s medium (Flow Laboratories) containing 10% (v/v) tryptose phosphate broth and 5% (v/v) calf serum (ETC) or 5% (v/v) rabbit serum (ETR) respectively.

**Viruses and virus assays.** The viruses used were three laboratory-established HSV-1 strains, HFEM (Watson et al., 1966), 17 (Brown et al., 1973) and KOS (Smith, 1964) and 11 recent local isolates, three HSV-2 strains, 186 (Rawls et al., 1968), 3345 (Sim & Watson, 1973) and G (Ejercito et al., 1968) and one strain each of BMV (Martin et al., 1966) and equine herpesvirus type 1 (EHV-1, strain RAC-H, Mayr et al., 1965; Thompson et al., 1976), two strains of pseudorabies virus (PRV, Kaplan & Vatter, 1959 and the strain isolated by Dr F. Dekking) and three HSV intertypic recombinants, BRS₅ and BRS₇ (Halliburton et al., 1980) and CSD (Morse et al., 1977). EHV-1 virus stocks and infected cell antigen preparations were produced in RK 13 cells. All other virus stocks and antigen preparations were prepared in BHK cells as described by Watson et al. (1966). Plaque titrations were performed by the suspension assay of Russell (1962) using an overlay medium containing CMC.

**Production of labelled infected cell antigen.** Confluent monolayers of BHK 21 or RK 13 cells in 80 oz roller bottles were infected at a multiplicity of infection (m.o.i.) of 5 to 10. After adsorption for 1 h at 37 °C the inoculum was decanted, the monolayers were washed and fresh ETC or ETR respectively added. At 4 h post-infection the medium was removed and the monolayers washed with growth medium lacking tryptose phosphate broth and containing either 1/10 the normal concentration of methionine (for radiolabelling with [35S]methionine at 2 μCi/ml) or 1/5 the normal concentration of glucose (for radiolabelling with [1,14C]glucosamine at 1 μCi/ml). The cells were then incubated for a further 16 h at 37 °C. At 20 h post-infection, cells were harvested, washed twice in phosphate-buffered saline (PBS), suspended in 3 ml PBS containing 1% NP40 and 0.5% sodium deoxycholate (DOC) per roller bottle and incubated at 4 °C for 1 h. After removal of the nuclei by low-speed centrifugation, the supernatant was centrifuged at 120000 g for 1 h. The supernatant was used in immunoprecipitation reactions and termed detergent-solubilized antigen.

**SDS–polyacrylamide gel electrophoresis.** Proteins were separated by SDS–PAGE followed by fixation, staining and autoradiography as described by Halliburton et al. (1977). Unless otherwise stated, the samples were heated at 100 °C for 2 min prior to electrophoresis.

**Production of antiserum to the oligomeric form of gB.** RK 13 cells were infected with HSV-1 (strain 17), incubated for 20 h and detergent-solubilized antigen was prepared as described above. Aliquots of the antigen were then centrifuged through 5 to 35% sucrose gradients containing 1% NP40, 0.5% DOC at 100000 g for 24 h at 4 °C, as described by Sarmiento & Spear (1979). The gradient was then fractionated and the fractions containing the oligomer (as detected by SDS–PAGE) were pooled, solubilized by addition of sample buffer (final concentrations, 0.05 M-Tris-Cl pH 7.0, 2% SDS, 5% 2-mercaptoethanol and 3% sucrose) and subjected to electrophoresis without prior heating of the sample. The protein band corresponding to the mature (fully glycosylated) oligomer of gB was located by Coomassie Brilliant Blue staining of excised strips of the gel and the unstained gel segment containing 2 to 5 μg of the upper oligomer was excised, emulsified in Freund’s incomplete adjuvant and injected into rabbits. Antiserum was prepared in rabbits by injection primarily and secondly in the footpad but subsequently in the nape of the neck at three- to four-weekly intervals. Bleeding was performed 10 days after the second and subsequent injections.

**Absorption of antisera.** Antiserum was made type-specific by absorption with excess heterologous antigen presented as monolayers of infected cells. The antigen was prepared by infecting monolayers of cells in 100 ml roller bottles (15 × 10⁶ cells) at an m.o.i. of 10 to 20. After adsorption at 37 °C for 1 h, the inoculum was decanted and replaced with fresh medium. At 20 h post-infection, infected cells were washed twice in cold PBS and stored at
4 °C. Antiserum (200 µl) was added to one bottle and incubated at 37 °C for 1 h, after which time the antiserum was transferred to a fresh bottle. This was repeated at 2 h and 4 h after the first addition. At 7 h after the first addition the antiserum was inactivated at 56 °C for 1 h and used in neutralization tests.

Agar gel immunodiffusion. Tests were performed with detergent-solubilized antigen in 2 to 3 mm layers of 1 % agar (Iongar, Oxoid) containing 0-1 % NP40 and 0-1 % sodium azide. After 24 to 48 h, gels were washed extensively in PBS containing 0-1 % NP40, dried and stained with acetic acid: methanol: water (9 : 9 : 2) containing 0-2 % Coomassie Brilliant Blue.

Neutralization tests. These were performed as described by Sim & Watson (1973) with some modifications. Samples of 4 × 10^4 to 1 × 10^5 p.f.u./ml of virus were incubated with an equal volume of antiserum appropriately diluted in PBS containing 5 % inactivated calf serum for the specific period. Surviving virus was then assayed by the suspension method of Russell (1962). All antisera were inactivated at 56 °C for 30 min prior to use. Results were presented as neutralization rate constants in the case of unabsorbed sera, or as percentage survivals relative to 100 % with preimmune serum in the case of absorbed sera.

Immunoprecipitation. Immunoprecipitates were obtained by incubating antiserum with detergent-solubilized antigen in appropriate proportions (usually 100 to 300 µl of antiserum to 100 µl antigen) at 4 °C for 48 h. The precipitate was pelleted through 0-5 ml 8 % sucrose in highly tapered glass tubes, washed six times by overlaying with ice-cold PBS, dissolved in 125 µl of sample buffer, disrupted by ultrasonication and the proteins separated by SDS-PAGE.

Iodination of protein. Na^125I was used to iodinate either immunoprecipitated polypeptides or segments of fixed and stained polyacrylamide gel containing the polypeptide to be analysed. In the latter case the gel segments were washed with 10 % methanol to remove SDS. Iodination was initially performed by the chloramine-T method (Elder et al., 1975; Littler et al., 1981) but subsequently by the method of Bolton & Hunter (1973). In the chloramine-T method, the iodination mixture contained 50 µg chloramine-T, 0-25 M-sodium phosphate buffer pH 7-5 and 200 µCi Na^125I (Amersham) in a total volume of 40 µl. The iodination of immunoprecipitated polypeptides was allowed to proceed for 30 to 45 s before addition of 0-5 ml sodium metabisulphite at 10 mg/ml to stop the reaction. Subsequent electrohoresis of these samples to separate the polypeptide allowed removal of excess ^125I at the gel front. In the iodination of gel segments, the reaction was allowed to proceed for 1 h prior to the addition of sodium metabisulphite. After 30 min, gel slices were washed several times with 10 % methanol to remove excess ^125I. Alternatively, radioiodination was done by treatment of gel slices washed in 0-2 M-borate buffer pH 8-5 and freeze-dried. One-hundred and twenty-five µCi Bolton and Hunter reagent [N-succinimidyl 3-(4-hydroxy 5-[125I]iodophenyl)propionate] was then added and the samples were left on ice for 2-5 h before the addition of 0-2 M-glycine (Bolton & Hunter, 1973). The gel slices were then washed extensively with 10 % methanol until the number of counts present in the wash was less than 10 % of the number incorporated.

Tryptic peptide analysis. Iodinated polypeptides were treated with 0-5 ml peroxidic acid for 1 h at 4 °C, freeze-dried and then digested overnight at 37 °C with 60 µg diphenylcarbamyl chloride-treated trypsin (Sigma) in 0-5 ml 50 mM-ammonium bicarbonate buffer pH 8-0. Samples were then repeatedly freeze-dried and resuspended in distilled deionized water to remove the ammonium bicarbonate and finally resuspended in 10 % acetic acid containing basic fuchsin.

Separation of tryptic peptides. Initially, tryptic peptides were separated by two-dimensional thin-layer chromatography on 200 × 200 × 0.25 mm silica G precoated chromatography sheets (Camlab, Cambridge, U.K.). The first dimension buffer was isobutanol: acetic acid: water (200 : 30 : 75) and the second dimension buffer was isobutanol: pyridine: acetic acid: water (15 : 10 : 3 : 12). Peptides were detected by autoradiography on Dupont Cronex film. Subsequently, tryptic peptides were separated by high-pressure liquid chromatography (HPLC) using an Applied Chromatography Systems Ltd HPLC system with Spherisorb-ODS C18 column from which peptides were eluted with gradients of 0 to 25 % isopropanol or 0 to 60 % methanol containing 0-1 M-NaH_2PO_4 and 0-1 % trifluoroacetic acid. ^125I-labelled peptides were detected by a Berthold Geiger-Müller detector.

RESULTS

Oligomeric glycoproteins of herpesviruses

Monolayers of BHK cells were infected with each of 12 strains of HSV-1 in the presence of [14C]glucosamine. Fig. 1 shows an autoradiogram of the glycoproteins separated by SDS-PAGE without prior heating of the sample. Apart from strain HFEM, all viruses produced two high molecular weight bands (about 160 000 mol. wt.) which are oligomeric forms of gB and pgB (Sarmiento & Spear, 1979; P. R. Kinchington & I. W. Halliburton, unpublished observations). Of the oligomers, the upper was always labelled more heavily than the lower. The two oligomers differed in mobility to a greater extent than gB and pgB and could differ slightly from strain to strain, particularly the lower oligomer (Fig. 1, compare strain 43 with 17 or 42). In contrast,
Fig. 1. [14C]Glucosamine-labelled infected cell polypeptides and oligomers specified by 12 strains of HSV-1. BHK cells in Linbro panels were infected at an m.o.i. of 10 and labelled from 4 to 12 h post-infection. Polypeptides were separated on a 9:25% polyacrylamide gel crosslinked with N,N'-diallyltartardiamide without heating of the samples at 100 °C prior to loading. The major HSV-1 glycoproteins are identified down the left-hand side together with the position of the two oligomeric bands (O) and the slower migrating oligomer (SO) of HFEM. Numbers or letters above each lane identify the virus strain. Strains 17 and HFEM are laboratory-established strains, the others being recent isolates.

HFEM produced no such oligomers (the only HSV strain not to do so of over 200 examined) but did have a very much slower mobility oligomer (Fig. 1) which varied in amount from experiment to experiment and in some cases could not be seen at all. In fact all other HSV strains did seem to produce very small amounts of a similar but not identical slower mobility oligomer.

Fig. 2 shows a comparison of the glycoprotein profiles of heated and unheated samples of HSV-1 strain 17 and HSV-2 strains 186 and 3345. Like HSV-1 strains other than HFEM, the HSV-2 strains synthesized two SDS-stable, heat-unstable oligomers but each type 2 oligomer was more highly mobile than the corresponding oligomer of HSV-1. Fig. 2 also shows heated and unheated samples of infected cell glycoproteins of three other herpesviruses, PRV (strains Dekking and Kaplan), EHV-1 and BMV. None of these viruses appeared to have oligomeric glycoproteins comparable to those of HSV although in the heated samples BMV specified 150K, 130K and 120K glycoproteins (marked with dots in Fig. 2) that were reduced in quantity in the unheated sample and the [14C]glucosamine-labelled material near the top of the EHV-1 profiles could have obscured oligomers.
Fig. 2. Glycoprotein profiles of BHK cells infected with strains of HSV-1, HSV-2, PRV, EHV-1, BMV or mock-infected. Monolayers of cells in Linbro panels were infected at an m.o.i. of 10 with HSV-1 strains 17 or HFEM, HSV-2 strains 186 or 3345, PRV strains Dekking (D) or Kaplan (K), EHV-1 strain RAC-H, or BMV (Martin et al., 1966) and labelled with [14C]glucosamine from 4 to 18 h post-infection. Samples were either treated in standard fashion with heating (H) at 100 °C for 2 min before loading onto an 8% polyacrylamide gel or were run without heating (U). Differences observed between heated and unheated sample lanes are indicated with arrows to the left. The major glycoproteins are identified with respect to HSV-1 or HSV-2.
Fig. 3. Agar gel immunodiffusion test of anti-oligomer serum against (a) antigen of HSV-1 strains KOS, 17 and HFEM and HSV-2 strains 3345, G and 186 and (b) antigen of HSV-1 strain 17, BMV, EHV-1 and mock-infected cells. The lines are stained with Coomassie Brilliant Blue. The break in the precipitin line between G and the AO serum is an artefact induced during drying of the gel.

Production and characterization of an antiserum to the oligomeric form of gB of HSV-1

An antiserum (hereafter referred to as anti-oligomer or AO serum) was prepared against the oligomeric form of gB of HSV-1 strain 17 as described in Methods and characterized by agar gel immunodiffusion, neutralization and immunoprecipitation. Fig. 3 shows the results of agar gel immunodiffusion. When reacted with detergent-solubilized extracts of cells infected with HSV-1 strains KOS, 17 or HFEM or with HSV-2 strains 3345, G or 186 the AO serum produced a single precipitin line common to both HSV-1 and HSV-2. This line was occasionally seen as a doublet, particularly when the antiserum was of high titre. The precipitin line was also present when HSV-detergent-solubilized antigen was reacted with HSV-1 general antiserum, supporting the conclusion that the line is virus-specific. No precipitin line was observed with mock-infected cell antigen. The line was difficult to detect when soluble antigen (Honess et al., 1974) was used instead of detergent-solubilized antigen, suggesting that the reacting component requires NP40 for solubilization. When reacted against BMV detergent-solubilized infected cell antigen at least one precipitin line was produced and when reacted against EHV-1 detergent-solubilized antigen at least two precipitin lines were produced one of which is a line of identity with that produced with HSV and with BMV (Fig. 3b).

Anti-oligomer serum neutralized HSV-1, HSV-2 and BMV (Fig. 4). From these results neutralization rate constants were determined (Sim & Watson, 1973) and are shown in Table 1. HSV-1 was neutralized at a two- to fourfold greater rate than HSV-2 and seven- to 16-fold greater rate than BMV. EHV-1 infectivity was not neutralized by the AO serum. In addition to common antibodies, antibodies specific to HSV-1 could also be detected after absorption of the anti-oligomer serum with excess HSV-2 or BMV antigen (Table 2). Furthermore, since absorption with HSV-2 antigen did not leave antibodies capable of neutralizing BMV, there is no neutralization site recognized by this antiserum present on BMV that is not also present on HSV-2. Similarly, following absorption with BMV antigen we can conclude that there is no neutralization site present on HSV-2 that is not also present on BMV.

Fig. 5 shows the proteins immunoprecipitated by the anti-oligomer serum from [14C]glucosamine-labelled detergent-solubilized antigen of cells infected with HSV-1 strains 17, KOS or
Table 1. Neutralization rate constants (K values) for HSV-1, HSV-2 and BMV by AO serum

<table>
<thead>
<tr>
<th>Expt. no.</th>
<th>Serum*</th>
<th>HSV-1 (HFEM)</th>
<th>HSV-1 (17)</th>
<th>HSV-2 (3345)</th>
<th>HSV-2 (186)</th>
<th>BMV</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>AO2a</td>
<td>0.098</td>
<td>0.035</td>
<td>0.031</td>
<td>0.041</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>AO2a</td>
<td>0.100</td>
<td>0.068</td>
<td>0.031</td>
<td>0.041</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>AO3a</td>
<td>0.040</td>
<td>0.010</td>
<td>0.024</td>
<td>0.006</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>AO3b</td>
<td>0.059</td>
<td>0.051</td>
<td>0.024</td>
<td>0.015</td>
<td></td>
</tr>
</tbody>
</table>

* The number after AO signifies the number of antigen injections prior to that bleed; a and b signify different rabbits.

Table 2. Neutralization of HSV-1, HSV-2 or BMV by AO serum absorbed with infected cell antigen

<table>
<thead>
<tr>
<th>AO serum absorbed with</th>
<th>HSV-1 (17)</th>
<th>HSV-2 (3345)</th>
<th>BMV</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSV-1 (17)</td>
<td>(109)*</td>
<td>(100)</td>
<td>(88)</td>
</tr>
<tr>
<td>HSV-2 (3345)</td>
<td>10</td>
<td>(106)</td>
<td>(150)</td>
</tr>
<tr>
<td>BMV</td>
<td>9</td>
<td>(87)</td>
<td>(87)</td>
</tr>
<tr>
<td>Mock-infected cells</td>
<td>1</td>
<td>3</td>
<td>9</td>
</tr>
</tbody>
</table>

* Values in parentheses do not differ significantly from 100%.

HFEM, BMV, HSV-2 strains 3345, G or 186 or EHV-1. With each of the HSV-1 or HSV-2 strains two glycoproteins corresponding in mobility to gB and pgB were precipitated although in some cases this appeared as a single band due to the amount precipitated. From cells infected with BMV at least five glycosylated species (molecular weights 150K, 130K, 120K, 53K and 47K) were precipitated by the antiserum. When reacted with EHV-1-infected cell antigen, three bands were seen (molecular weights 138K, 100K and 87K) although the 138K glycoprotein is present in very small amounts and is not apparent in Fig. 5. However, if the pellet precipitated by the anti-oligomer serum was labelled in vitro with 125I (as described in Methods) prior to
Cross-reacting herpesvirus glycoprotein

SDS–PAGE then the 138K glycoprotein was easily detected. Clearly, however, the anti-oligomer serum precipitated antigenically related glycoproteins from cells infected with HSV-1, HSV-2, BMV and EHV-1.

It should be noted that the amount of gB precipitated can vary with different antigen preparations and the apparent low efficiency of precipitation of gB from the HFEM extract in Fig. 5(a) was not a reproducible finding. However, the fact that, relatively speaking, only small amounts of pgB were precipitated did seem to be reproducible. With HSV-2 there were in addition small quantities of lower molecular weight (e.g. 45K and 30K) proteins precipitated. These may be analogous to the components reported for gB by Pereira et al. (1981) and Balachandran et al. (1982) which have been shown by the latter workers to contain subsets of gB peptides.

Tryptic peptide analysis of gB and antigenically related glycoproteins

The polypeptides immunoprecipitated from HSV-1, HSV-2, BMV and EHV-1 detergent-solubilized antigen by the anti-oligomer serum were next subjected to tryptic peptide analysis. In the initial experiments they were labelled by the chloramine-T method of Elder et al. (1977) as modified by Littler et al. (1981), digested with trypsin and the resulting soluble peptides separated by two-dimensional chromatography.

Fig. 6 shows the results obtained with gB of HSV-1 strains KOS, 17 (the oligomeric band from purified virus) and HFEM, of HSV-2 strains 186 and 3345 and of intertypic recombinants C5D, RB50 and RB52 and the 120K glycoprotein precipitated by the anti-oligomer serum from BMV-infected cells. Also shown for comparative purposes are gC of HSV-1 strain 17 obtained from purified virus, bovine serum albumin (BSA) and a blank gel segment all removed from SDS–polyacrylamide gels. Firstly, no obvious spots were found from gel segments containing no protein. The large dark area seen at the top right of this and of all other maps in Fig. 6 is either free iodine or may be the non-specific acrylamide gel contaminant that was found to be iodinated by Elder et al. (1977). Secondly, although the number of peptides produced from the three different proteins gC, BSA and gB were about the same (25, 30 and 24 to 32 respectively), the three polypeptides clearly showed characteristically different peptide fingerprints. However, the fingerprints of the polypeptides immunoprecipitated by the anti-oligomer serum from cells infected with each of the three HSV type 1 strains, the three type 2 strains or with BMV were all extremely similar to each other. Most of the major radioiodinated peptides were identical although there was one peptide missing (marked with an arrow) with HSV-1 strain KOS but present in all other cases and some of the minor peptides seemed to vary in quantity. Most of these variations were not, however, reproducible and these antigenically related polypeptides therefore seemed to be very conserved in structure.

Also shown in Fig. 6(b) are the tryptic peptide fingerprints for gB of three HSV intertypic recombinants. C5D was isolated from mixed infections of HFEM and 186 (Morse et al., 1977) and RB50 RB52 from mixed infections of HFEM and 3345 (Halliburton et al., 1980). The majority of tryptic peptides, as expected, did not differ from those of either parent and although some of the minor species were not readily detected, the results strengthen the conclusion that the peptides of HSV-1 and HSV-2 strains as detected by this method are extremely similar. However, it can be very difficult to reproduce precisely the same experimental conditions for this method and, as will be obvious from a study of Fig. 6, comparison of fingerprints is very difficult. In addition, since resolution of the peptides by another method might differ and therefore give more data on similarities and/or differences, we decided to try separation of the peptides by HPLC. Initially the immunoprecipitated proteins were labelled by the chloramine-
Fig. 6. Tryptic peptide analysis of (a) gC of HSV-1 strain 17 purified virus, BSA, a gel segment with no protein (np) and the polypeptide immunoprecipitated by the AO serum from detergent-solubilized extracts of cells infected with KOS or BMV (the 120K glycoprotein) or the oligomer of gB from purified strain 17 virus [17 (PV)O]. (b) gB immunoprecipitated by the AO serum from detergent-solubilized extracts of cells infected with HFEM, 186, C5D, 3345, RB50 or RB52. Gel slices containing the
polypeptides were iodinated with $^{125}$I by the chloramine-T method, digested in situ with trypsin, the soluble peptides eluted and resolved by two-dimensional chromatography on silica thin-layer chromatography sheets. The arrow with KOS indicates the position of a major peptide present in all polypeptides immunoprecipitated by anti-oligomer serum but missing with KOS.
T method prior to separation by SDS–PAGE which facilitated removal of much of the excess $^{125}$I before digestion with trypsin. The tryptic peptides were then separated by HPLC by elution from a Spherisorb-ODS C18 column using gradients of 0 to 25% isopropanol.

Some difficulty was, however, observed in precise reproduction of the data partly due to the chloramine-T iodination process and partly due to the isopropanol gradient elution system. The polypeptides were therefore labelled by the Bolton and Hunter iodination procedure as described in Methods and were eluted from the HPLC column by gradients of 0 to 60% methanol. Fig. 7 shows the profiles obtained under these conditions with gB of HSV-1 strain HFEM, gB of HSV-2 strain G and a co-run of gB and pgB of strain G. The three profiles were essentially identical and these results were reproducible in different runs using either the same or different HSV strains. If pgB was run alone, however, minor differences from gB were observed in the small peaks at the hydrophobic (left-hand) end of the gradient. These may be related to the different degrees of glycosylation of gB and pgB. Fig. 8 shows a comparison of the profiles for a polypeptide immunoprecipitated from BMV-, EHV-1- and HSV-1-infected cells. In general terms the profiles were again very similar to each other with regard to the number, size and position of peaks. There were, however, some minor differences particularly in the magnitude of peaks for the BMV or EHV-1 polypeptides relative to the HSV-1 gB profile, two of the more prominent differences being arrowed in Fig. 8. These differences may be due to slight alteration of amino acid sequence or structural conformation of the peptide in question facilitating additional binding of $^{125}$I to available amide groups compared to the situation with HSV gB.

More detailed analysis of these profiles with regard to the number of peaks, precise position of elution (to within 1% methanol concentration) and relative magnitude revealed that of a total of 20 peaks present and identical in all of the HSV gB profiles studied (HFEM, 17 and KOS of HSV-1 and G, 186 and 3345 of HSV-2), 17 of these were present and identical in the BMV 120K (and 130K profile, data not shown) profile and 14 were present and identical in the EHV-1 138K profile. As can be seen from the data in Fig. 8, apart from those marked, the differences were not in major peptides. Finally, to show that unrelated polypeptides did give completely different profiles by this method, Fig. 9 shows the results for HSV-2 strain 186 gB, IgG and rabbit serum albumin (RSA). It is clear that the number, the magnitude and the relative position of peaks differ for unrelated proteins.

DISCUSSION

Cross-reactions between herpesviruses have been studied by a variety of approaches. Several studies have demonstrated relationships between HSV-1, HSV-2 and BMV, both genetically with the BMV genome showing 14% base sequence homology (Sterz et al., 1973/4) and the HSV-2 genome showing 47% base sequence homology with HSV-1 (Kieff et al., 1972; Sugino & Kingsbury, 1976) and antigenically by complement fixation, immunodiffusion, immunofluorescence, immunoprecipitation and neutralization (Sterz et al., 1973/4; Killington et al., 1977, 1978; Norrild et al., 1978; Yeo et al., 1981). However, HSV-1 shows less than 5% base sequence homology with EHV-1 (Ludwig et al., 1971) and although antigenic cross-reactions between HSV-1 and EHV-1 have been demonstrated by complement fixation, gel diffusion, immunofluorescence and immunoprecipitation (Plummer, 1964; Blue & Plummer, 1973; Killington et al., 1977; Yeo et al., 1981) the viruses do not cross-neutralize. More recently, Davison & Wilkie (1983) examined the regions of the HSV-1 and HSV-2 genomes to which EHV-1, PRV and varicella-zoster virus DNA would hybridize and concluded that these herpesviruses possessed several highly conserved genes. Attempts to identify specific cross-reacting polypeptides between herpesviruses have shown that an antiserum to gB (Ag-11) of HSV-1 cross-reacts with HSV-2 and BMV by immuno-electrophoresis and by neutralization (Norrild et al., 1978) and antiserum to gD (Band II) of HSV-1 also neutralizes HSV-2 and BMV (Killington et al., 1978). In addition, the major cross-reacting protein of HSV-1, HSV-2, BMV, PRV and EHV-1 by immunoprecipitation has been identified as a major DNA-binding protein of each virus (Littler et al., 1981; Yeo et al., 1981) and tryptic peptide analysis of this cross-reacting protein of all five viruses has shown structural similarities (Littler et al., 1981). Recently, Gibson et al. (1984) found significant homology between two Epstein–Barr virus
Fig. 7. Tryptic peptide analysis of gB of (a) HSV-1 strain HFEM, (b) HSV-2 strain G and (c) a co-run of gB and pgB of HSV-2 strain G. The polypeptides were immunoprecipitated by anti-oligomer serum and separated by SDS-PAGE. Gel slices containing the polypeptides were iodinated with Bolton and Hunter reagent, digested \textit{in situ} with trypsin and the peptides separated by HPLC on a Spherisorb-ODS C18 column with elution by gradients of 0 to 60% methanol.
Fig. 8. Tryptic peptide analysis of polypeptides immunoprecipitated by anti-oligomer serum from detergent-solubilized extracts of cells infected with BMV, EHV-1 or HSV-1 HFEM. Details are as for Fig. 7. Prominent differences from results obtained with HFEM are indicated by arrows.

Fig. 9. Tryptic peptide analysis of gB of HSV-2 strain 186, IgG or rabbit serum albumin (RSA). SDS-polyacrylamide gel slices containing the polypeptides were processed as described in the legend to Fig. 7.

reading frames and the HSV-1 and HSV-2 140K and 38K proteins which are associated with ribonucleotide reductase activity. Such findings may well reflect conservation of function and suggest that conserved proteins such as these have central roles in virus replication. We have shown in this report using an antiserum produced in rabbits against the purified oligomeric form of HSV-1 gB that HSV-1, HSV-2 and BMV possess common neutralization sites and also, together with EHV-1, common antigenic sites involved in immunoprecipitation. The antiserum immunoprecipitates gB and pgB of HSV-1 and HSV-2, glycoproteins of estimated molecular weights 150K, 130K, 120K, 53K and 47K of BMV and glycoproteins of estimated molecular weights 138K, 100K and 87K of EHV-1. The major BMV glycoproteins precipitated probably correspond to those of 155K, 133K and 125K reported by Norrild et al. (1978) to be precipitated by monospecific antisem to Ag-11. The relationship of the 53K and 47K glycoproteins to the others is not known. The 138K, 100K and 87K EHV-1 glycoproteins precipitated could...
correspond to the 138K (VP10), 90K (VP13) and 87K (VP14) glycoproteins of the Army 183 strain of EHV-1 reported by Turtinen & Allen (1982).

Tryptic peptide analysis of gB and pgB of the three HSV-1 and three HSV-2 strains studied, of BMV glycoproteins of 120K and 130K molecular weight and of the EHV-1 glycoprotein of 138K molecular weight indicates a high degree of structural homology between each of them. The tryptic peptide profiles obtained after separation of peptides either by two-dimensional chromatography or by HPLC are very similar to each other but markedly different from the corresponding profiles of unrelated proteins such as gC, BSA, RSA or IgG. Certainly, one would expect the fingerprints of gB and pgB (formerly identified as gA) of the same virus to be very similar since the genes specifying the glycoproteins co-map on the genome (Marsden et al., 1978; Ruyechan et al., 1979; Halliburton, 1980) and the glycoproteins are antigenically related and for some time now have been thought to be two forms of a single polypeptide probably differing only in the degree of processing or glycosylation (Eberle & Courtney, 1980b). Balachandran et al. (1982) have also shown that an anti-HSV-2 monoclonal antibody against the gAB complex and an anti-gAB rabbit serum each immunoprecipitates gB and pgB (gA) from extracts of infected cells, and they concluded from tryptic peptide mapping of gB and gA that the two glycoproteins share the same protein backbone. The minor differences found in this study between the fingerprints of gB and pgB in the small peaks at the hydrophobic end of the HPLC profile could be related to different degrees of glycosylation. Studies on the effect of digestion of gB with endo-β-N-acetylglucosaminidase H (Endo H) to remove high-mannose oligosaccharides followed by tryptic peptide analysis have not, however, facilitated identification of the glycosylated peptides. This is possibly due to minor alteration of the hydrophobicity of the peptide by removal of only a small oligosaccharide chain, resulting in no apparent alteration in the position of elution of the peak in the HPLC tryptic peptide profile.

The similarities of the tryptic peptide profiles of gB of HSV-1 and HSV-2 strains is consistent with recent sequencing data which shows that major portions (80% or more) of the gB gene of HSV-1 and HSV-2 share about 90% nucleotide and amino acid homology (Bzik et al., 1984; D. Bzik, B. Fox, S. Warner & S. Person, personal communication). The structural homology of the 130K and 120K BMV polypeptides suggests that BMV may specify a series of glycoproteins analogous to gB and pgB of HSV-infected cells and is consistent with the reported antigenic relatedness of BMV and HSV. The results for the EHV-1 138K polypeptide are perhaps somewhat less expected since previous studies have reported a lower degree of antigenic homology between this virus and HSV-1, HSV-2 or BMV. The fact that the anti-gB oligomer serum does not neutralize EHV-1 infectivity and that slightly fewer tryptic peptides are conserved in the HPLC g138K profiles is perhaps a reflection of this situation. Nevertheless, EHV-1 does specify polypeptides which are antigenically and structurally related to HSV gB.

The work of Davison & Wilkie (1983), however, on the homology between herpesvirus genomes demonstrated by molecular hybridization that the EHV-1 genome contains several regions which are homologous to regions of the HSV genome. One of these includes a section of the EHV-1 genome that hybridizes to the HSV-1 BamHI G fragment and to the HSV-2 BamHI B fragment which encode several genes including that for gB. It is therefore conceivable that there is some conservation of the nucleotide sequences encoding gB and hence some conservation of amino acid sequence and antigenicity as described in this study. It is also possible that the conservation of structure of antigenically related glycoproteins between HSV-1, HSV-2, BMV and EHV-1 is correlated with conservation of some essential function. Glycoprotein B of HSV-1 has been reported to be essential for productive virus infection and to play a role in penetration of virus particles into the host cell (Sarmiento et al., 1979; Little et al., 1981). Future studies involving other herpesviruses will demonstrate whether or not they also specify polypeptides related to gB of HSV and studies currently in progress with mutants of some of the above viruses may help to clarify the essential role of gB in virion infectivity.

This work was supported by a Medical Research Council project grant. B.W.S. is a holder of a Medical Research Council Studentship and P.R.K. of a Science Research Council Studentship. We would like to thank Dr E. Littler for his advice, Professor D. H. Watson for his encouragement and Mrs C. I. Moorhouse for typing this manuscript.
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Cross-reacting herpesvirus glycoprotein


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(Received 7 August 1984)