

Herpes Simplex Virus Glycoprotein gA/B: Evidence that the Infected Vero Cell Products Comap and Arise by Proteolysis

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We recently reported (Pereira et al., Proc. Natl. Acad. Sci. U.S.A. 78:5202-5206, 1981) that herpes simplex virus 1 and 2 glycoproteins, previously designated gA and gB, could not be differentiated by a bank of independently derived type-specific and type-common monoclonal antibodies. We also reported that from lysates of infected Vero cells, all but one monoclonal antibody precipitated gA/B glycoproteins which had faster electrophoretic mobility than the corresponding infected HEP-2 cell glycoproteins and a set of three small polypeptides which we designated g(A+B) reactive polypeptides 1, 2, and 3. Antibody H368, the single exception, failed to react with the gA/B glycoproteins or related antigens accumulating in infected Vero cells. In this paper, we report the following results. (i) The high-apparent-molecular-weight gA/B glycoproteins accumulating in infected HEP-2 cells were cleaved by a proteolytic enzyme contained in Vero cell lysates to yield more rapidly migrating proteins that were indistinguishable from authentic Vero cell gA/B glycoproteins. Like its authentic counterpart, the cleaved gA/B glycoproteins failed to react with H368 monoclonal antibody. In addition, the lysate cleaved HEP-2 cell gA/B glycoproteins into g(A+B) reactive polypeptides 2 and 3. (ii) The proteolytic activity contained in the uninfected cell lysates was inhibited by *N*- α -*p*-tosyl-L-lysine chloromethyl ketone and is therefore trypsin-like. (iii) Pulse-chase experiments indicated that the cleavage of gA/B glycoproteins occurred during or soon after translation but that the accumulation of g(A+B) reactive polypeptide 1 was a consequence of a delayed processing event. (iv) Analysis of herpes simplex virus 1 \times herpes simplex virus 2 recombinants indicated that the determinants of type-specific immune reactivity and electrophoretic mobility of gA/B glycoproteins and g(A+B) polypeptides map near the right terminus of herpes simplex virus 1 BamHI-G.

Herpes simplex virus 1 (HSV-1) virions and membranes of infected cells were initially reported to form, on electrophoresis in denaturing polyacrylamide gels, three bands containing high-molecular-weight glycosylated proteins. These glycoproteins were designated virion proteins VP7, VP7.5, and VP8 (4, 5, 16). Subsequently, on the basis of pulse-chase studies, the glycosylated proteins were designated gC, gB, and gA, respectively (15). However, recent reports from this and other laboratories indicate that gA and gB glycoproteins are related, inasmuch as both reacted with monospecific antisera prepared against each glycoprotein (2) or with banks of independently derived monoclonal antibodies (10). In the course of studies with the monoclonal antibodies, it was noted that gA/B glycoproteins made in Vero cells migrated significantly more rapidly than those made in HEP-2 cells. Furthermore, the infected Vero cell

lysates contained three relatively small proteins which were precipitated by 23 of 24 monoclonal antibodies reactive with gA/B glycoprotein. The proteins were designated g(A+B) reactive polypeptides. The g(A+B) polypeptides of HSV-1 (F) had apparent molecular weights of 40,000, 39,000, and 29,000 and differed in electrophoretic mobility from those made by HSV-2 (G), which were 41,500, 37,000, and 27,000 in apparent molecular weight. The exception, monoclonal antibody H368, reacted with glycoprotein gA/B of HSV-2 made in HEP-2 cells but not with the gene product accumulating in Vero cells. These observations led to the suggestion that the differences in electrophoretic mobility of the glycoprotein gA/B made in Vero and HEP-2 cells could reflect differences in glycosylation or cleavage.

In this paper, we report that the electrophoretic mobility of glycoprotein gA/B very likely

reflects the cleavage of the glycoprotein gene product in Vero cells, inasmuch as exposure of glycoprotein gA/B made in HEP-2 cells to uninfected Vero cell extracts resulted in proteolytic cleavage and yielded products indistinguishable from those accumulating in infected Vero cells. We also reinforce the conclusion that gA and gB glycoproteins and the g(A+B) reactive polypeptides are related by showing that they comap within the same restricted region of HSV DNA.

MATERIALS AND METHODS

Viruses and cells. Isolation and properties of HSV-1 (mP), HSV-1 (F), and HSV-2 (G) strains and the derivation and properties of recombinants RH1G7, RH1G8, RH1G13, RH1G44, and RH1G48 were described previously (1, 3, 6, 7). Human epidermoid carcinoma 2 (HEp-2) cells and African green monkey kidney (Vero) cells were grown in Earle minimum essential medium supplemented with 10% fetal calf serum. After infection with virus, cells were maintained in medium containing 1% serum.

Monoclonal antibodies to HSV. Hybridomas producing monoclonal antibodies to HSV glycoproteins were derived from fusion of BALB/c MOPC 21 NS-1 myeloma cells with spleen cells of BALB/c mice immunized with HSV. Procedures used for selection and characterization of hybridomas were published elsewhere (9a, 11). Properties of H233 and H368 monoclonal antibodies to glycoproteins gA and gB were previously described (10).

Preparation of radiolabeled infected cell extracts. HEp-2 or Vero cells, as indicated in the text, were infected with 10 PFU per cell and labeled with [³⁵S]methionine (50 μ Ci/ml, >400 Ci/mmol; New England Nuclear Corp., Boston, Mass.) at 8 to 18 h postinfection. For pulse-chase experiments, infected cells were incubated in medium without methionine at 5 to 6 h postinfection. The cells were labeled for 15 min with [³⁵S]methionine (100 μ Ci/ml) (pulse). Duplicate cultures replenished with medium containing unlabeled methionine were incubated for an additional 3 h (chase). Lysates of infected cells were prepared by incubating the cells on ice for 30 min in phosphate-buffered saline containing 1% Nonidet P-40 and 1% sodium deoxycholate. The antigen lysates were centrifuged for 60 min at 25,000 rpm and 4°C in a Beckman SW27.1 rotor.

Immunoprecipitation and polyacrylamide gel electrophoresis. Radiolabeled antigens were mixed with mouse ascites fluids (25 to 50 μ l) containing monoclonal antibodies. After incubation at room temperature for 60 min, rabbit anti-mouse immunoglobulin G (Miles Laboratories, Inc., Elkhart, Ind.), followed by protein A-Sepharose (Sigma Chemical Co., St. Louis, Mo.), were added to precipitate the antigen-antibody complexes. Immune precipitates adsorbed to protein A-Sepharose were washed repeatedly in cold phosphate-buffered saline containing 0.1% Nonidet P-40 and 1% sodium deoxycholate.

Denatured samples were subjected to electrophoresis in 9.25% polyacrylamide gels cross-linked with *N,N*-diallyltartardiamide and containing sodium dodecyl sulfate. Procedures for solubilization of the pro-

teins, electrophoresis, and autoradiography were described previously (9). Reagents used for polyacrylamide gel electrophoresis were purchased from Bio-Rad Laboratories, Richmond, Calif.

Incubation of infected HEp-2 cell lysates with uninfected Vero cell extracts. Uninfected Vero cell extracts were prepared from confluent cell sheets. The cells were harvested and incubated in phosphate-buffered saline containing 1% Nonidet P-40 and 1% sodium deoxycholate at 4°C for 30 min. Extracts were centrifuged as described above. Radiolabeled infected HEp-2 cell lysates were mixed with equal volumes (usually 25 μ l) of uninfected Vero cell extracts and allowed to react for 60 min at room temperature. In some experiments, 10^{-5} M *N*- α -*p*-tosyl-L-lysine chloromethyl ketone (TLCK), a trypsin inhibitor (14), or L-1-tosylamide-2-phenylethyl chloromethyl ketone (TPCK), a chymotrypsin inhibitor (13), were added to HEp-2 infected cell lysates before addition of the uninfected Vero cell extracts. The final concentration of either TLCK or TPCK was 10^{-5} M. To obtain partial cleavage products of gA/B glycoprotein, uninfected Vero cell extracts were allowed to react with infected HEp-2 cell lysates. Monoclonal antibody H233 (25 μ l) was added at different times to stop the reaction, followed by protein A-Sepharose to precipitate the immune complexes. TLCK and TPCK were purchased from Sigma Chemical Co.

Incubation of infected Vero cells with proteolytic inhibitors. Infected Vero cells were incubated with 10^{-6} M TLCK or TPCK in the medium at 7 h postinfection and labeled with [³⁵S]methionine at 8 to 18 h postinfection in the presence of proteolytic inhibitors.

RESULTS

Electrophoretic mobility of proteins precipitated from infected HEp-2 and Vero cells by monoclonal antibody to glycoproteins gA/B after a pulse and after a chase. One unresolved question is whether the event underlying the difference in electrophoretic mobility of gA/B glycoprotein made in infected HEp-2 and Vero cells occurred during or immediately after translation or during delayed processing of the newly translated protein. To differentiate between these two possibilities, we compared the glycoprotein gA/B reactive antigens, labeled as described in Materials and Methods, in infected Vero and HEp-2 cells after a 15-min pulse and after a chase. Autoradiograms of the immune precipitated polypeptides electrophoretically separated in denaturing polyacrylamide gels are shown in Fig. 1. The results showed the following.

(i) The event responsible for the difference in electrophoretic mobility of gA/B glycoproteins made in infected Vero and HEp-2 cells occurs during or soon after translation, inasmuch as the gA/B glycoproteins labeled during the pulse reflected the difference in electrophoretic mobility reported previously (10).

(ii) The g(A+B) reactive polypeptides 2 and 3

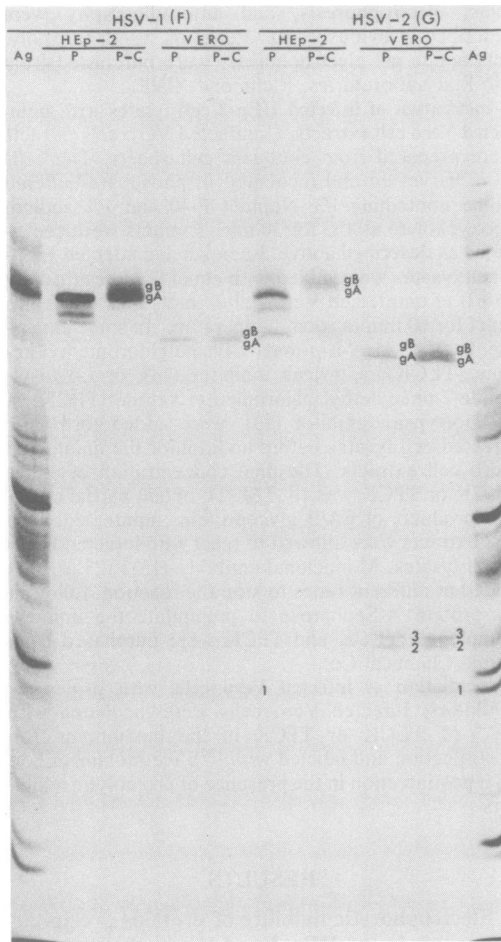


FIG. 1. Autoradiographic images of electrophoretically separated [³⁵S]methionine-labeled polypeptides immunoprecipitated with monoclonal antibody H233. HEP-2 and Vero cells were infected with HSV-1 (F) and HSV-2 (G) and radiolabeled during a 15-min pulse at 6 h postinfection as described in the text. 1, 2, and 3, g(A+B) reactive polypeptides; gA and gB, more slowly migrating forms of the glycoproteins; P, pulse; P-C, pulse-chase; Ag, labeled antigens present in infected-cell lysates available for reaction with antibody.

are also produced during or soon after translation, inasmuch as both polypeptides 2 and 3 were present in immune precipitates obtained with lysates of pulse-labeled infected cells. It should be noted that, although the HSV-1 g(A+B) reactive polypeptides were too faint to appear in the prints of the autoradiograms, they were uniformly present in the lysates of pulse-labeled, HSV-1-infected Vero cells. The HSV-1 and HSV-2 g(A+B) reactive polypeptide 1 was detected during the chase but not after the pulse. In addition, we consistently noted a change in

electrophoretic mobility of polypeptide 3 after the chase. Therefore, the electrophoretic mobility of glycoproteins gA/B and the accumulation of polypeptides 2 and 3 reflect a translational or rapid post-translational event. The presence and mobility of polypeptides 1 and 3 as well as of the fully processed forms of gA/B glycoproteins reflect slow post-translational events.

Cleavage of glycoprotein gA/B made in HEP-2 cells by proteolytic enzymes contained in extracts

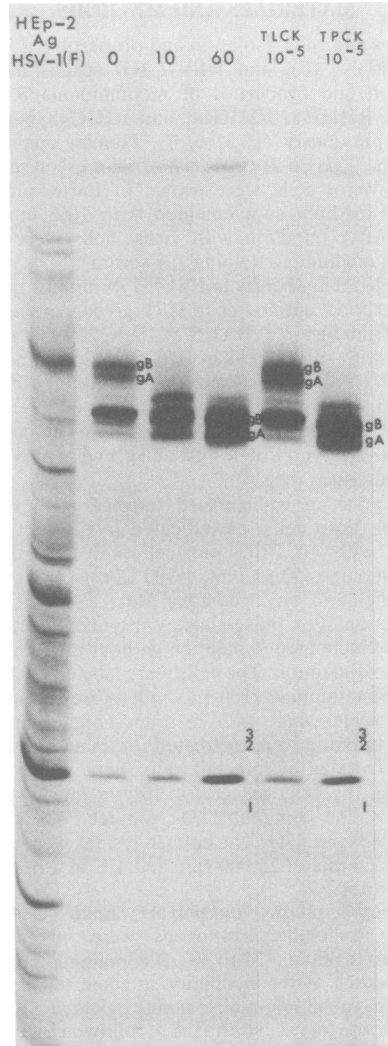


FIG. 2. Autoradiographic images of electrophoretically separated [³⁵S]methionine-labeled polypeptides immunoprecipitated with H233 monoclonal antibody from HSV-1 (F)-infected HEP-2 cell lysates. Uninfected Vero cell extracts were mixed with infected HEP-2 cell lysates and incubated for 0, 10, or 60 min. TLCK or TPCK was added to infected HEP-2 cell lysates to a final concentration of 10^{-5} M before mixing with Vero cell extracts.

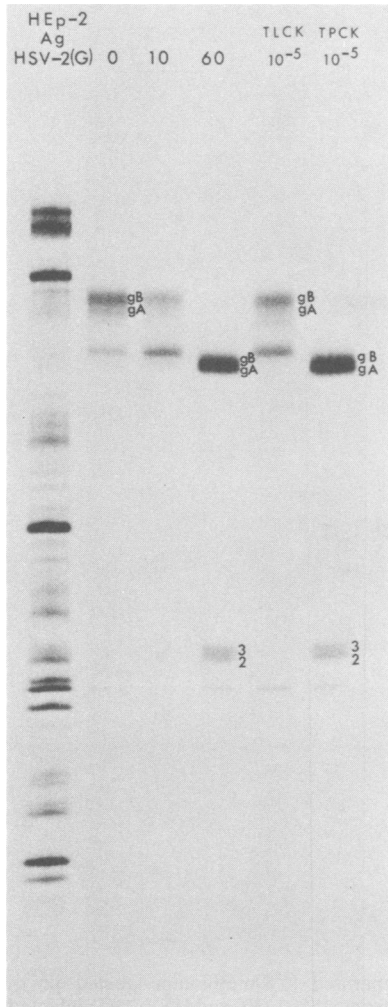


FIG. 3. Autoradiographic images of electrophoretically separated [³⁵S]methionine-labeled polypeptides immunoprecipitated with H233 monoclonal antibody from HSV-2 (G)-infected HEP-2 cell lysates treated as described in the legend to Fig. 2.

of uninfected Vero cells. One hypothesis which could explain the difference in electrophoretic mobility of Vero and HEP-2 cell gA/B glycoproteins is that the nascent polypeptide is rapidly cleaved by a host cell enzyme. To test this hypothesis, equal portions of infected HEP-2 cell lysates were mixed with uninfected Vero cell extracts prepared as described in Materials and Methods. At intervals after mixing, the gA/B glycoproteins were precipitated with monoclonal antibody to the glycoproteins and subjected to electrophoresis in denaturing polyacrylamide gels. Limited cleavage of gA/B glycoprotein made in HEP-2 cells was detected 10 min after exposure to uninfected Vero cell

extracts (Fig. 2 and 3). The cleavage was complete after 60 min. In addition to the more rapidly migrating forms of glycoproteins gA/B, the autoradiograms revealed the presence of g(A+B) reactive polypeptides 2 and 3 but not of 1.

To determine the nature of the proteolytic activity responsible for the cleavage, the mixtures were treated with TLCK and TPCK. The cleavage was inhibited by TLCK but not by TPCK (Fig. 2 and 3), suggesting that the proteolytic activity is trypsin-like with respect to amino acid specificity at the cleavage site. Furthermore, TLCK prevented cleavage not only in vitro but also in infected cells. Addition of TLCK 1 h before the addition of [³⁵S]methionine to infected Vero cells precluded cleavage of glycoprotein gA/B (Fig. 4).

In a previous report (10), we showed that, whereas 23 of 24 independently derived monoclonal antibodies reacted with gA/B glycopro-

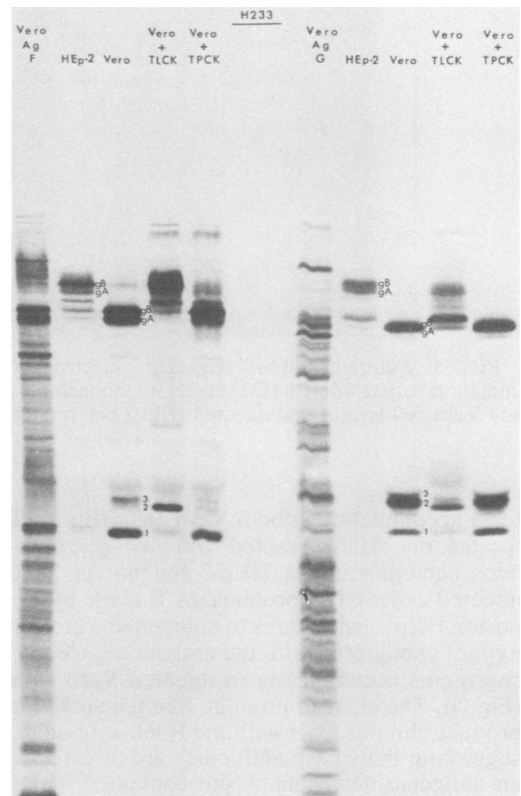


FIG. 4. Autoradiographic images of electrophoretically separated [³⁵S]methionine-labeled polypeptides immunoprecipitated with H233 monoclonal antibody from HSV-1 (F)- and HSV-2 (G)-infected Vero cells treated with 10⁻⁶ M TLCK or TPCK present during infection before preparation of infected Vero cell lysates.

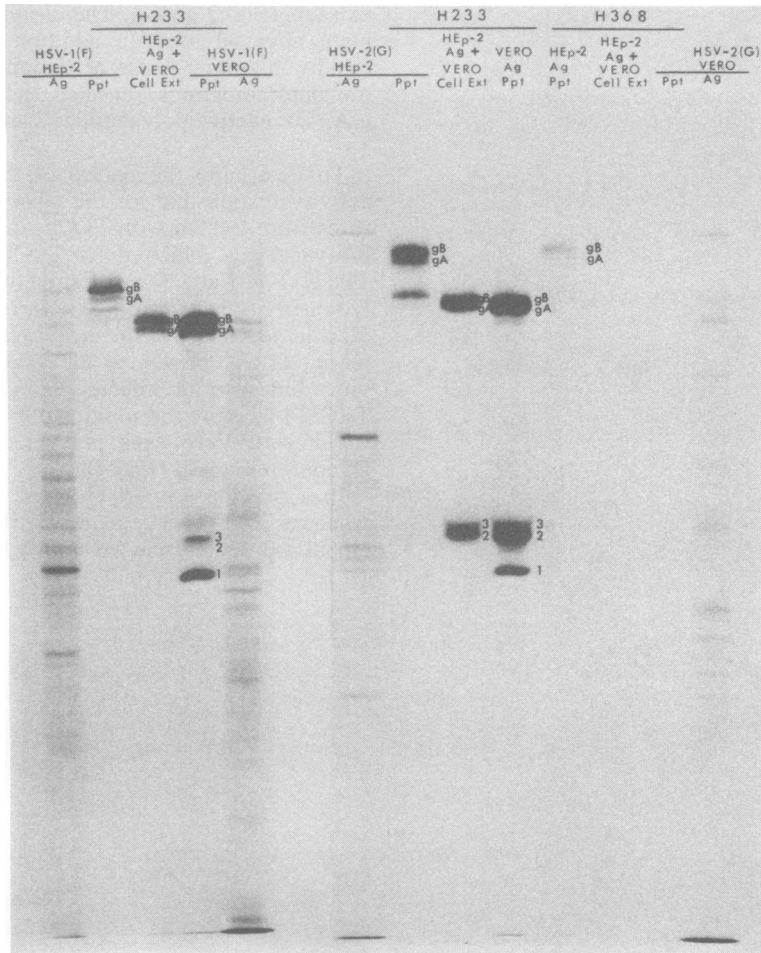


FIG. 5. Autoradiographic images of electrophoretically separated [35 S]methionine-labeled polypeptides immunoprecipitated with H233 and H368 monoclonal antibodies from HSV-1 (F)- and HSV-2 (G)-infected HEP-2 and Vero cell lysates and infected HEP-2 cell lysates mixed with extracts of uninfected Vero cells.

teins accumulating in both Vero and HEP-2 cell lysates, one, H368, reacted with gA/B glycoproteins accumulating in HEP-2 but not in Vero infected cells. Glycoproteins gA/B made by exposing HEP-2 cell lysates to uninfected Vero cell extract comigrated with the authentic gA/B glycoproteins accumulating in infected Vero cells (Fig. 5). The cleaved product, like the authentic product, did not react with the H368 antibodies, suggesting that these antibodies are directed to an antigenic determinant site contained within the degraded or lost portion of the molecule.

Fine mapping of glycoproteins gA/B and g(A+B) reactive polypeptides. On the basis of electrophoretic mobilities of the glycoproteins specified by HSV-1 \times HSV-2 recombinants, the glycoproteins gA and gB were mapped in a region of the HSV genome bounded by coordinates 0.30 to

0.42 map units (12). More recent studies narrowed the coordinates of the gA/B genes to DNA *Bam*HI fragment G (8). This fragment maps between 0.337 and 0.388 map units. The experiments described below and concerned with more precise mapping of gA/B genes were done for two reasons. First, the narrowest map coordinates available do not exclude the possibility that the glycoproteins gA/B are specified by independent genes. The second reason concerned the g(A+B) reactive polypeptides. Previous studies have shown that g(A+B) reactive HSV-1 polypeptides differ in electrophoretic mobility from HSV-2 and that all monoclonal antibodies which precipitated glycoproteins gA/B from infected Vero cell lysates also precipitated the g(A+B) reactive peptides (10). Unambiguous demonstration that glycoprotein gA/B is a

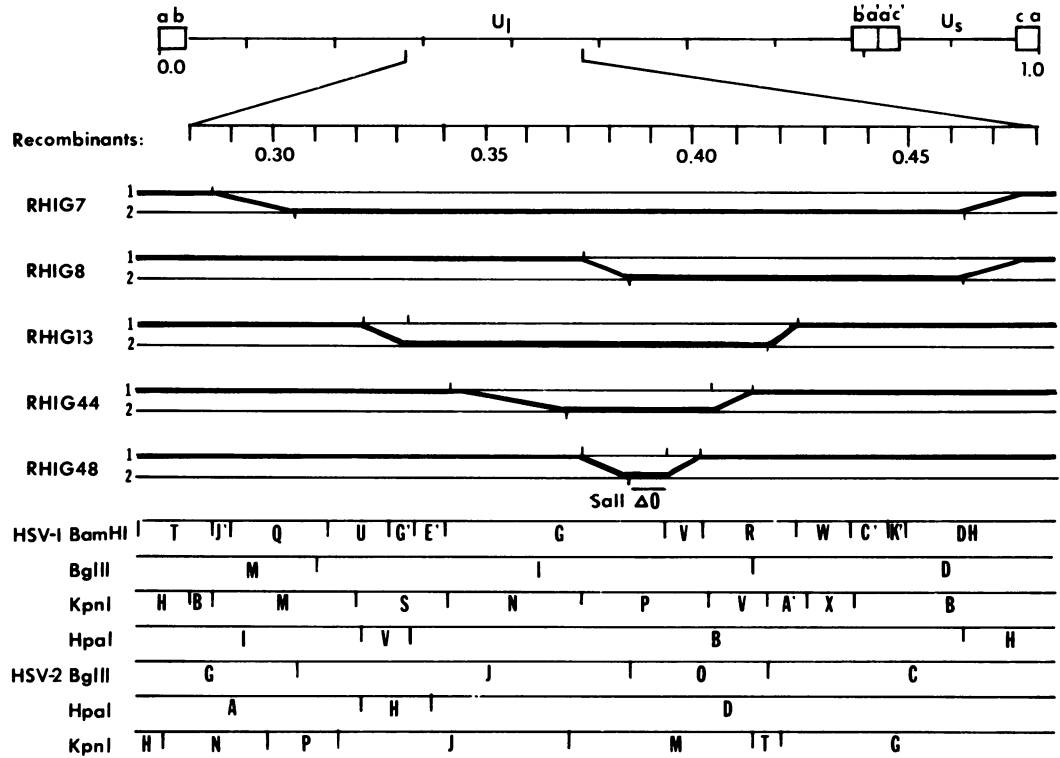


FIG. 6. Restriction endonuclease maps of HSV-1 × HSV-2 recombinants RH1G7, RH1G8, RH1G13, RH1G44, and RH1G48 produced by marker rescue of HSV-1 (mP) *tsHA1* with HSV-2 (G).

precursor of the g(A+B) reactive polypeptides requires evidence that the DNA sequences specifying the glycoprotein gA/B polypeptides and g(A+B) reactive peptides comap. The mapping of the DNA sequences specifying gA/B and g(A+B) took advantage of the availability of a set of suitable HSV-1 × HSV-2 recombinants produced by marker rescue of HSV-1 (mP) *tsHA1* with HSV-2 (G) DNA and monoclonal antibodies which react with either HSV-2 glycoproteins gA/B (e.g., H368) or with both HSV-1 and HSV-2 glycoproteins gA/B (e.g., H233).

Figure 6 shows the maps of crossover sites in the DNAs of recombinants RH1G7, RH1G8, RH1G13, RH1G44, and RH1G48 described in detail by Conley et al. (1). The map of the DNA of each recombinant is shown within the confines of a double line representing HSV-1 and HSV-2 DNAs (designated 1 and 2, respectively). The heavy line represents the DNA of the recombinant. The diagonal lines linking the lines representing HSV-1 and HSV-2 DNAs represent the approximate crossover sites. The crossover sites are based on the restriction endonuclease maps for HSV-1 (mP) and HSV-2 (G) DNAs shown in the lower portion of the figures.

The coordinates of the crossover sites differ slightly from those published by Conley et al. (1), largely because the availability of cloned DNA fragments permitted a more precise alignment of the various restriction enzyme maps. As previously established, the HSV-2 sequences substituting HSV-1 sequences in RH1G7 have, as their maximum and minimum left boundaries, the restriction enzyme cleavage sites HSV-1 *BamHI*-T-J' and HSV-2 *BglII*-G-J, respectively; the maximum and minimum right boundaries are the HSV-2 *KpnI*-G-D and HSV-1 *HpaI*-B-H restriction enzyme cleavage sites, respectively. The left maximum and minimum boundaries of the HSV-2 sequences in RH1GH8 are defined by HSV-1 *KpnI*-N-P and HSV-2 *BglII*-J-O cleavage sites, respectively, whereas the right maximum and minimum boundaries are identical to those of RH1G7 DNA. The maximum and minimum left boundaries of HSV-2 sequences in RH1G13 are HSV-1 *HpaI*-I-V and -V-B cleavage sites, respectively; the maximum and minimum right boundaries are HSV-2 *BglII*-O-C and HSV-1 *BamHI*-R-W cleavage sites, respectively. In recombinant RH1G44, the maximum and minimum left HSV-2 boundaries are contained be-

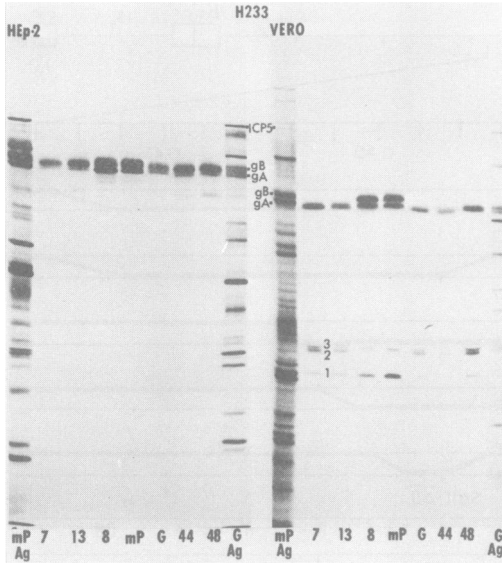


FIG. 7. Autoradiographic images of electrophoretically separated [35 S]methionine-labeled polypeptides immunoprecipitated with cross-reacting H233 monoclonal antibody from lysates of HEp-2 and Vero cells infected with recombinants and parent strains.

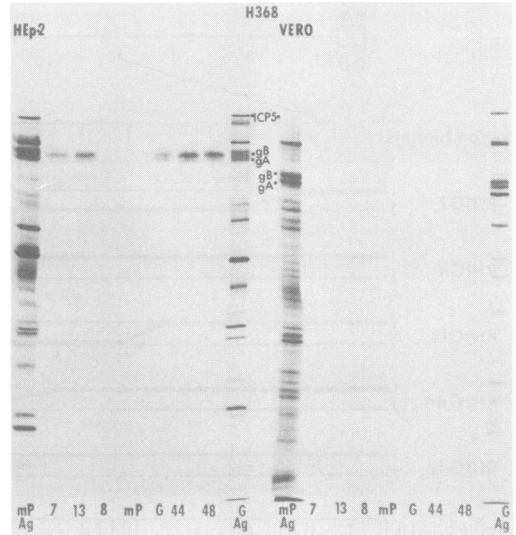


FIG. 8. Autoradiographic images of electrophoretically separated [35 S]methionine-labeled polypeptides immunoprecipitated with type 2-specific H368 monoclonal antibody from lysates of HEp-2 and Vero cells infected with recombinants and parent strains.

tween HSV-1 *KpnI*-J-M cleavage sites, whereas the right maximum and minimum boundaries are contained between the HSV-1 *Bam*HI-R-W and HSV-1 *KpnI*-P-V cleavage sites. In the recombinant RH1G48, the left maximum and minimum boundaries of the HSV-2 sequences are identical to the corresponding boundaries of RH1G8 DNA; the right maximum and minimum boundaries are contained between HSV-1 *Bam*HI-G-V and HSV-1 *Bam*HI-V-R cleavage sites. Also shown is the map location of HSV-1 *Sal*I fragment Δ O, which was shown to rescue the *ts*HA1 mutation in marker rescue tests (1).

Figures 7 and 8 show the autoradiographic images of the [35 S]methionine-labeled glycoproteins gA/B precipitated with monoclonal antibodies H233 and H368, respectively, from lysates of Vero and HEp-2 cells infected with parent and recombinant virus strains. Monoclonal antibody H233, as previously reported, precipitated both HSV-1 and HSV-2 glycoproteins gA/B from Vero and HEp-2 cell lysates and g(A+B) reactive polypeptides from infected Vero cell lysates. H368 monoclonal antibody reacted only with the HSV-2 glycoproteins gA/B produced in HEp-2 cells. On the basis of electrophoretic mobility and reactivity with monoclonal antibody, the glycoproteins gA/B and g(A+B) reactive polypeptides made by RH1G8 resemble those of the HSV-1 (mP) parent, whereas the glycoproteins gA/B and g(A+B) reactive polypeptides of all other recombinants resemble

those of the HSV-2 parent. Results of neutralization tests with monoclonal antibodies H233 and H368 and the RH1G series of recombinants are shown in Table 1. The results are in accordance with immunoprecipitation reactions, in that type 2-specific monoclonal antibody H368 neutralized the HSV-2 (G) parent and recombinants RH1G7, RH1G13, RH1G44, and RH1G48 but failed to neutralize the HSV-1 parent and recombinant RH1G8.

Two features of the results shown in Fig. 6 through 8 are of significance. First, the g(A+B) reactive polypeptides generated in infected Vero cells by HSV-1 (mP) differ from those generated by HSV-1 (F), reinforcing the conclusion that

TABLE 1. Plaque reduction tests with H233 and H368 monoclonal antibodies, intertypic recombinants, and parental strains

Strain	PFU of virus surviving/total PFU of virus (%) ^a	
	H233	H368
RH1G7	0/110 (0)	38/110 (34.5)
RH1G8	0/235 (0)	226/235 (96.2)
RH1G13	0/233 (0)	83/233 (35.6)
RH1G44	0/313 (0)	83/313 (26.5)
RH1G48	0/200 (0)	37/200 (18.5)
HSV-1 (mP)	0/121 (0)	115/121 (95.0)
HSV-2 (G)	0/228 (0)	49/228 (21.5)

^a Average results of duplicate tests.

the g(A+B) products are virus specific. The second aspect concerns the physical map location of the sequences specifying the HSV-2 antigenic determinants in the RH1G series of recombinants. The RH1G series of recombinants were selected by marker rescue of HSV-1 (mP) *tsHA1* with HSV-2 (G) DNA, and those shown in Fig. 7 and 8 specify HSV-2-infected cell polypeptide 8. Furthermore, the temperature-sensitive mutation was rescued by a small cloned fragment (*SaII*- Δ O) which selects from infected cells mRNA capable of translating in vitro the infected cell polypeptide 8. The temperature-sensitive mutation is, therefore, closely linked to the gene specifying polypeptide 8. The results shown in Fig. 6 through 8 indicate that the sequences specifying the antigenic determinant sites for gA/B and g(A+B) are in close proximity to the gene specifying polypeptide 8, inasmuch as the HSV-2 antigenic determinants are expressed by all of the recombinants except RH1G8. Since RH1G8 and RH1G48 overlap, the most probable sequence encoding the antigenic determinant sites is within a DNA region bounded by HSV-1 *KpnI*-N-P and the HSV-2 *BglII*-J-O cleavage sites. This region contains the left crossovers of both RH1G8 and RH1G48. Since the sequences specifying the gA/B antigenic determinant sites are to the left of those specifying polypeptide 8, it is likely that the left crossover of RH1G8 is to the right of that of RH1G48.

DISCUSSION

Derivation and structure of the g(A+B) reactive polypeptides. The results presented in this report indicate that polypeptides indistinguishable from authentic g(A+B) reactive polypeptides 2 and 3 can arise by cleavage of glycoproteins gA/B made in HEp-2 cells by uninfected Vero cell extracts and suggest that the authentic g(A+B) polypeptides arise by a similar mechanism. The g(A+B) reactive polypeptide 1 and the more rapidly migrating forms of polypeptide 3 are products of processing taking place significantly later. All three polypeptides retain a major portion of antigenic determinant sites of the gA/B glycoproteins, even though they are considerably smaller in molecular weight. These conclusions are based on the following observations. (i) The g(A+B) reactive polypeptides share antigenic determinant sites with glycoproteins gA/B. (ii) g(A+B) reactive polypeptides are not host gene products, inasmuch as their electrophoretic mobility in denaturing polyacrylamide gels is genetically determined by the virus, and the phenotypic properties of g(A+B) reactive polypeptides and of gA/B glycoproteins comigrate within a narrow region of the HSV

genome. (iii) The g(A+B) reactive polypeptides contain a significant fraction of the major antigenic determinants of glycoproteins gA/B, even though they represent somewhat less than 30% of the apparent molecular weight of the gA/B glycoproteins. This conclusion is based on the observation that all g(A+B) reactive polypeptides were precipitated by 23 of 24 independently derived monoclonal antibodies to glycoproteins gA/B. These monoclonal antibodies comprised 11 which neutralized the virus and 12 which did not. The exception was a monoclonal antibody (H368) which immunoprecipitated HSV-2 (G) glycoproteins gA/B made in HEp-2 but not those made in infected Vero cells. If the frequency of emergence of monoclonal antibodies per fractional length of the polypeptide reflected the density of antigenic determinant sites, it could be concluded that g(A+B) reactive polypeptide 1 from both HSV-1 and HSV-2 (apparent molecular weights, 29,000 and 27,000, respectively) contained a major portion of the type-common antigenic determinant sites of the parent gA/B glycoprotein molecule.

Location of the sequences specifying antigenic determinant sites of gA/B glycoproteins and g(A+B) reactive antigens on the physical map of the HSV genome. The availability of the RH1G series of HSV-1 \times HSV-2 recombinants (1) permitted us to map the gene location of the sequences specifying the antigenic determinant sites of glycoproteins gA/B within a small portion of the region to which the gA and gB genes were previously assigned. Specifically, the earliest studies assigned the infected cell polypeptide 11 (9) and gA and gB genes (12) to 0.3 to 0.42 map units. In more recent studies, it was shown that the HSV-1-infected cell polypeptide 11 mapped at the right end of HSV-1 *BamHI* fragment G, immediately to the left of the gene specifying polypeptide 8. Because of the variability in electrophoretic patterns of glycoproteins gA/B reported earlier (12), it was not clear that the polypeptide 11 actually corresponds to glycoproteins gA/B, even though the genes specifying these polypeptides have consistently mapped in the same location. In this report, we show that the antigenic determinants of glycoproteins gA/B, as identified by immunoprecipitation with both type-common (H233) and HSV-2-specific (H368) monoclonal antibodies, map within *BamHI*-G to the right of the *KpnI*-N-P cleavage site but to the left of *SaII* fragment Δ O (Fig. 6).

Cleavage of glycoproteins gA/B. The significant observation presented in this report is that glycoproteins gA/B made in infected HEp-2 cells were cleaved by uninfected Vero cell extracts to yield two sets of products. These were polypeptides which comigrated with authentic gA/B

glycoproteins made in infected Vero cells and g(A+B) reactive polypeptides which comigrated with authentic g(A+B) reactive polypeptides seen in infected Vero but not in HEp-2 cell lysates. Our results suggest that both products are generated by cleavage of the gA/B precursor by a trypsin-like proteolytic enzyme. Several aspects of our results are of particular interest, specifically the following.

(i) We did not observe complete conversion of the gA/B glycoproteins made in infected HEp-2 cells into g(A+B) reactive polypeptides by cleavage with the enzyme contained in the uninfected Vero cell lysate. Similarly, both gA/B and g(A+B) reactive polypeptides accumulate in infected Vero cells even after a long chase. These observations suggest that only a portion of the gA/B precursor is cleaved to yield g(A+B) reactive polypeptides, and therefore, the cleavage sites which generate the g(A+B) reactive polypeptides must be inaccessible in some of the precursor molecules. Nothing is known regarding the fate of the peptides cleaved from gA/B precursor to generate the authentic infected Vero cell gA/B glycoproteins and the g(A+B) reactive polypeptides. Inasmuch as detection of such putative peptides requires antibody and since all but one monoclonal antibody were found to react with g(A+B) reactive polypeptides, we have no immunological reagents with which to determine whether the cleaved peptides are conserved or degraded in infected Vero cells.

(ii) The cleavage of gA/B precursor to yield the g(A+B) reactive polypeptides 2 and 3 and the gA/B molecules which accumulate in infected Vero cells must occur during or soon after the synthesis of the precursor, inasmuch as the products of the reaction were present in the lysates of cells harvested immediately after a 15-min pulse. In contrast, the g(A+B) reactive polypeptide 1 was not detected until after a chase. The proteolytic event which generates this polypeptide appears to occur later in the life of the gA/B polypeptide. The nature of the enzyme involved is not known.

(iii) The demonstration that the difference in electrophoretic mobilities is due to cleavage of the protein rather than to differences in the extent of glycosylation of glycoproteins gA/B in infected Vero and HEp-2 cells is of considerable significance. It indicates that differences in electrophoretic mobilities of HSV glycoproteins commonly observed when viruses are grown in different cell lines must be interpreted with caution, inasmuch as they may be due to partial proteolysis or to both cleavage and differential glycosylation.

(iv) Because the size of the virus yield from infected Vero cells is as high as or higher than

that obtained from infected HEp-2 cells, the function of gA/B glycoproteins may not be affected by the proteolysis involved in generation of the authentic Vero infected cell gA/B glycoproteins. Whether g(A+B) reactive polypeptides are capable of expressing any of the functions of gA/B glycoproteins is currently unknown.

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