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Characterization of the 92,000-Dalton Glycoprotein Induced by Herpes Simplex Virus Type 2

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Evidence is presented showing that the 92,000-dalton glycoprotein (g92K) induced by herpes simplex virus (HSV) type 2 has properties distinct from those assigned to any other HSV glycoprotein. First, the carbohydrate composition and extent of sulfation differ from those of glycoproteins D and E. Second, two clonally unrelated monoclonal antibodies, AP1 and LP5, shown in this paper to specifically immunoprecipitate g92K, do not react with any of the known processed forms of glycoproteins B, C, D, and E. Third, by using HSV type 1/HSV type 2 intertypic recombinants and a simple radioimmunoassay, the target antigen of the two monoclonal antibodies was shown to map in the same region as g92K (0.846 to 0.924). Fourth, the intertypic recombinant R12-3 was shown by sodium dodecyl sulfate-polyacrylamide gel electrophoresis of infected cells to induce the HSV type 2 g92K and HSV type 1 gD and gE, whereas R12-1, which did not induce g92K, induced HSV-2 gE and an altered gD, providing genetic evidence that g92K is encoded, at least in part, by a different region of the genome from that encoding gD and gE.

Considerable progress has been made toward identification and characterization of the glycoproteins encoded by herpes simplex virus (HSV) types 1 (HSV-1) and 2 (HSV-2). HSV is known to induce at least four glycoproteins. Spear identified gB, gC, and gD (32), and gE was identified by Bauke and Spear (3). Balachandran and co-workers identified a glycoprotein in HSV-2-infected cells of apparent molecular weight 75,000 which they designated gF (1, 2) and which has recently been shown to be antigenically related to HSV-1 gC (gC-1) (26, 36, 37). Based upon these results and to simplify the nomenclature, it was suggested at a recent International Herpesvirus Workshop (held from 31 July to 5 August 1983 in Oxford, England) that the earlier designations of g75K and gF should be replaced by gC-2. Accordingly, we have used gC-2 throughout this paper. Glycoproteins B, C, D, and E have been shown to be distinct from each other by one or more of the following techniques: immune precipitation with monoclonal antibodies, tryptic peptide fingerprinting, or physical mapping (1, 2, 12, 15, 18, 21, 25, 26, 28, 31).

While mapping gB and gD (designated 117K and 51K in that study), Marsden et al. (21) described two additional HSV-2-induced glycoproteins, g63K and g92K. Although not yet rigorously established, g63K is probably gC-2. This paper is concerned with g92K. We report the identification of two monoclonal antibodies which react with g92K. We mapped the coding region for the target antigen and performed radioisotopic labeling and genetic experiments which showed that g92K has properties which distinguish it from those assigned to identified glycoproteins.

MATERIALS AND METHODS

Cells. BHK21 clone 13 cells (19) were used throughout.

Virus. HSV-1 strain 17 *syn*$^+$ (4) and HSV-2 strain HG52 (33) were used in this study. The isolation of HSV-1/HSV-2 intertypic recombinants and the determination of their genome structure have been previously reported (5, 9, 15, 20, 21, 29).

Radioactive labeling. Confluent monolayers in 30- or 50-mm-diameter dishes were infected at a multiplicity of infection of 10 to 20 PFU per cell. After 1 h unadsorbed virus was removed, and the infected cell monolayer was labeled for the times indicated below with (i) ^{35}S inorganic sulfate, (ii) [^{35}S]methionine, (iii) [^{3}H]mannose, or (iv) [^{14}C]glucosamine as described by Hope et al. (15). The monolayers were harvested for either sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (21) or immunoprecipitation (22).

Production of monoclonal antibodies, immunoprecipitation, and radioimmunoassay. Hybridoma cell lines secreting monoclonal antibodies were produced, and monoclonal antibodies AP1 and LP5 were identified by immunoprecipitation as described by McLean et al. (22). The monoclonal antibody gB2, which precipitates both the HSV-1- and HSV-2-induced gB, was obtained from M. Levine. The antibody specific for gC-1 (designated 1001) was described by Palfreyman et al. (24). Control ascites fluid was produced from mice injected with the parental myeloma cell line P3X63Ag8.

The radioimmunoassay system used in this system was based on the method of Colombatti and Hilgers (8). Briefly, microtiter plates (Titertek; Flow Laboratories, Irvine, Scotland) were sensitized with virus (either a reference strain or a recombinant) by passive absorption of virus, diluted in 25 μl of phosphate-buffered saline (PBS), and incubated overnight at 37°C. After incubation, the plates were washed twice with PBS, and excess binding sites were blocked by a 1-h incubation at 37°C with 200 μl of PBS containing 0.5% bovine serum albumin (BSA) per well. Ascites fluid, diluted 20-fold in PBS containing 0.1% BSA (PBS-BSA), was added (25 μl), and the plates were incubated at 37°C for 1 h followed by washing three times with PBS-BSA. ^{125}I-labeled protein A (25 μl), ca. 2.5 ng per well diluted in PBS-BSA, was then added. After a final 30-min incubation, the plates were washed three times with PBS. Bound ^{125}I-protein A was eluted with 5 M NaOH, and radioactivity was estimated by gamma counting.

Gel electrophoresis. Samples were prepared, and SDS-PAGE was carried out by using the buffer system of Laemmli (17) and either 5 to 12.5% gradient gels cross-linked

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with 1 in 20 (wt/wt) *N,N'*-methylenebisacrylamide (BIS) (22) or 7.5% gels cross-linked with 1 in 50 (wt/wt) *N,N'*-methylenebisacrylamide (21). Immunoprecipitated polypeptides were resuspended in a buffer containing 0.05 M Tris-hydrochloride (pH 6.8)–2% SDS–5% 2-mercaptoethanol–10% glycerol and boiled for 5 min before electrophoresis.

Fluorography. Gels were treated with En^{3}Hance (New England Nuclear Corp., Boston, Mass.), dried, and exposed to Kodak X-Omat XS1 film.

Polypeptide nomenclature. For glycoproteins, we have used the nomenclature adopted at the International Conference on Human Herpesviruses, Atlanta, Ga. (7), and the Eighth International Herpesvirus Workshop, Oxford, England. Where we cannot or do not wish to distinguish between different forms of a glycoprotein, we have written out the prefix in full, e.g., glycoprotein B. Glycoprotein A (32) was shown to be antigenically related to gB for both HSV-1 (10) and HSV-2 (27). These glycoproteins are variously referred to in the literature as gA/B, gA/gB, or, as in this manuscript, simply as gB.

RESULTS

Carbohydrate and inorganic sulfate composition of g92K. g92K had been identified previously only on the basis of its incorporation of [^{14}C]glucosamine (21). The incorporation of other radioactively labeled precursors is shown in Fig. 1. Under the conditions used, the 92,000 (92K)-dalton glycoprotein labeled heavily with glucosamine, only moderately with mannose, and poorly, if at all, with inorganic sulfate

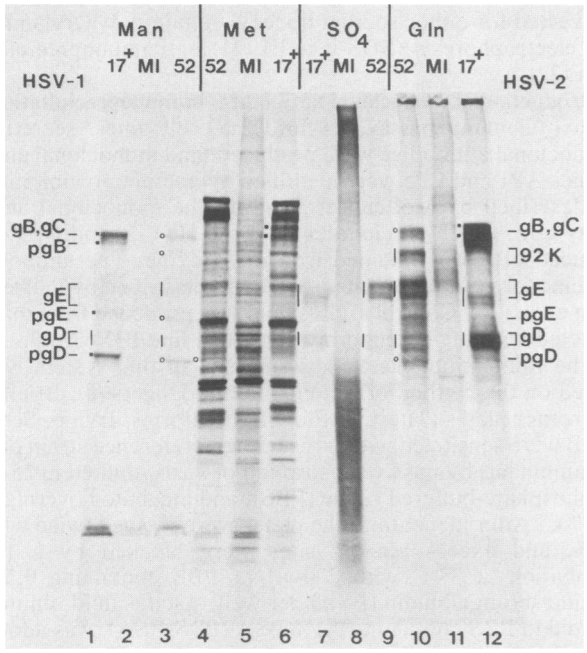

FIG. 1. Comparison of the polypeptides of infected and uninfected cells labeled with [^{3}H]mannose (Man), [^{35}S]methionine (Met), ^{35}S-inorganic sulfate (SO_4), or [^{14}C]glucosamine (Gln). Cells were infected with either 17 *syn*$^+$ (17$^+$) or HG52 (52) or were mock-infected (MI). Labeling with mannose was from 5 to 6.5 h after infection and with the other isotopes from 2 to 24 h after infection. Polypeptides were separated by electrophoresis in a 5 to 12.5% SDS–polyacrylamide gel. In this figure the symbols for HSV-1 glycoproteins (■) and HSV-2 glycoproteins (○) are placed adjacent and to the left of the appropriate protein.

(lanes 10, 3, and 9). These combined labeling characteristics distinguished g92K from gD and gE. In Fig. 1, g92K appears as a doublet (lane 10). There is no readily identifiable HSV-1 equivalent for this glycoprotein (lane 12) (21), although a possible candidate is suggested below.

Immunoprecipitation with monoclonal antibodies AP1 and LP5. Two monoclonal antibodies, AP1 and LP5, were isolated which immunoprecipitated a glycoprotein with an apparent molecular weight of 92K. Confirmation that these antibodies react with g92K and that g92K represents an immunologically distinct species was obtained from isotopic labeling and mapping experiments. The reactions of AP1 and LP5 with extracts from HSV-1- and HSV-2-infected cells are shown in Fig. 2 and 3. In Fig. 2, cells were labeled with [^{14}C]glucosamine, and extracts from HSV-1- and HSV-2-infected cells were prepared as described (lanes 1, 8, and 16). AP1 reacted with a g92K polypeptide and to a lesser extent with a high-molecular-weight band (upper arrow) from HSV-2-infected cells (lanes 10 and 15) but not with any polypeptide from HSV-1-infected cells (lane 3). Polypeptides of similar molecular weight were not precipitated by monoclonal antibodies specific for gB (lanes 2 and 9), gC-1 (compare lane 4 with lane 11), gE-2 (compare lane 5 with lanes 12, 18, and 20), or gC-2 (compare lane 6 with lanes 13, 17, and 19). Control precipitations with normal mouse serum are shown in lanes 7, 14, and 21. Collectively, these data demonstrate that AP1 reacts with an HSV-2 polypeptide of molecular weight 92K which is distinct from gB, gC, and gE.

Both AP1 and LP5 specifically immunoprecipitated a [^{35}S]methionine-labeled HSV-2 92K polypeptide (Fig. 3, lanes 2, 3, 6, and 7) which was present in only trace amounts in the extract (lanes 1 and 5). Several other proteins (e.g., 157K, the major capsid protein) were precipitated by these antisera, but not specifically since they were also precipitated by the control ascites fluid (lanes 4 and 8). When [^{14}C]glucosamine was employed as label, AP1 and LP5 specifically precipitated g92K (lanes 10, 11, 14, and 15). They similarly immunoprecipitated a [^{3}H]mannose-labeled polypeptide with an apparent molecular weight of 92K from HSV-2-infected cells (lanes 18 and 19), demonstrating that g92K contains both glucosamine and mannose. Three other bands were sometimes precipitated by AP1 and LP5 from glucosamine-labeled (upper two arrows, lanes 14 and 15) or glucosamine- and mannose-labeled polypeptides (lower arrow, lanes 14, 15, 18, and 19). These polypeptides are present in the precipitates in much lower amounts than g92K, and their relationship to g92K remains to be established. Comparison of the 3- to 9-h results with the 3- to 26-h results (compare lane 10 with lane 14 and lane 11 with lane 15) suggested that g92K is synthesized in increased amounts late in infection.

Mapping the target antigen of AP1 and LP5. Extracts of uninfected or infected cells were bound to microtiter wells and incubated with antibody, and the amount of bound antibody was estimated with ^{125}I-protein A. The interaction of AP1 and LP5 with 14 recombinant viruses is shown in Table 1. Also shown is the binding of a control ascites fluid and the type-common anti-gD monoclonal antibody 1140 (24). Antibody 1140 will react with cells in which the infection has progressed to at least synthesis of gD. The data shown in Table 1 allowed the recombinants to be unambiguously classified with respect to interaction with AP1 and LP5. Correlation of these data with the genome structures of the recombinants (Fig. 4) gave the coordinate limits, 0.846 to 0.950, for the target antigen. This position is delimited on the left by B × 1(28-1-1) (the HSV-1 *Bam*HI q-m′ site in IR_S) and

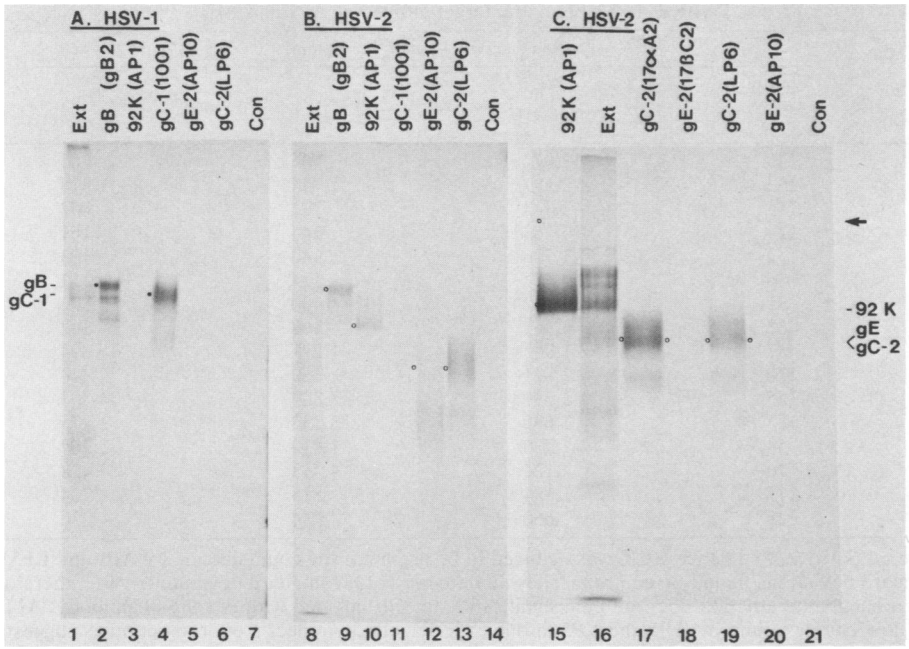

FIG. 2. Immunoprecipitation of polypeptides by monoclonal antibodies. BHK cells were infected with HSV-1 strain 17 *syn*$^+$ (panel A, lanes 1 to 7) or HSV-2 strain HG52 (panels B and C, lanes 8 to 21) and labeled with [^{14}C]glucosamine from 2 to 19 after adsorption. Panels A and B are from one experiment and panel C is from another. An extract of the infected cells (Ext, lanes 1, 8, and 16) was used for immunoprecipitation with the following sera: type common anti-gB (gB2, lanes 2 and 9); HSV-1-specific anti-gC (1001, lanes 4 and 11); HSV-2-specific anti-92K (AP1, lanes 3, 10, and 15); HSV-2-specific anti-gE (AP10, lanes 5, 12, and 20; 17βC2, lane 18); HSV-2-specific anti-gC (LP6, lanes 6, 13, and 19; 17αA2, lane 17); and control normal mouse serum (Con, lanes 7, 14 and 21). Polypeptides were separated by electrophoresis in 7.5% SDS–polyacrylamide gels. The glycoproteins against which the antisera are directed are indicated above the tracks. Symbols: ◖, minor bands precipitated by AP1 and LP5; ■, HSV-1 glycoproteins; ○, HSV-2 glycoproteins. The specificity of AP10 for gE and of LP6 for gC-2 was established by two criteria with monoclonal antibodies 17αA2 and 17βC2, which are specific for gC-2 and gE, respectively (1, 2). The first criterion was the similar electrophoretic mobility of the precipitated polypeptides (compare lane 17 with lane 18 and lane 19 with lane 20) and the second was the map position obtained by radioimmunoassay (data not shown).

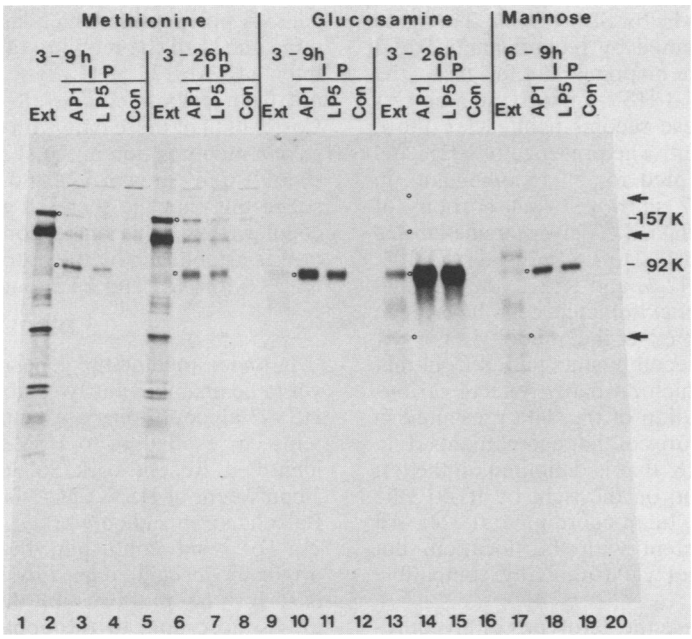

FIG. 3. Immune precipitation of the 92K protein from HSV-2-infected cells labeled with [^{35}S]methionine, [^{14}C]glucosamine, and [^{3}H]mannose. The infected cells were labeled for the times indicated, and an extract was made (Ext) which was used for immunoprecipitation (IP) with two anti-92K monoclonal antibodies (AP1 and LP5) and a control ascites fluid (Con). Polypeptides were separated by electrophoresis in a 5 to 12.5% SDS–polyacrylamide gel. Symbols: ◖, minor bands precipitated by AP1 and LP5; ○, HSV-2 glycoproteins.

TABLE 1. Mapping the target antigen of AP1 and LP5

Virus	Radioactivity (counts per 10 min)				Binding of AP1 and LP5[a]
	Control ascites fluid	1140 (anti-gD)	AP1	LP5	
17 *syn*$^+$ (HSV-1)	143	12,072	65	65	−
HG52 (HSV-2)	162	2,587	1,744	1,818	+
B × 1(28-1-1)	115	2,343	734	802	+
RD104	124	16,863	144	161	−
RD113	152	3,776	140	103	−
RD213	136	9,387	124	51	−
RE4	91	8,782	100	76	−
RE6	261	4,801	168	162	−
R12-3	57	1,905	1,271	894	+
R12-5	170	2,811	120	46	−
17$^+$ × 11^r	155	2,231	3,810	4,381	+
B × 1(24)	185	1,655	3,232	2,775	+
D × 1(34-1)	58	730	1,010	1,267	+
D × 1(34-2)	76	7,124	4,413	4,428	+
D × 1(48)	117	1,154	815	704	+
F × 9(5-8)	125	660	434	230	+[b]

[a] Binding was considered positive, and hence g92K was deduced to be present if the counts bound by AP1 and LP5 were greater than the mean counts plus 2 standard deviations bound by control ascites fluid (mean = 133, standard deviation = 49.2; therefore, >231 is positive).

[b] Although in this experiment antibody LP5 does not bind significantly to cells infected with F × 9(5-8) antibody, AP1 does, as does LP5 in other experiments. The low counts bound by AP1 and LP5 in this experiment may reflect a poor infection as suggested by the low counts bound by the control anti-gD monoclonal antibody (1140).

on the right by RE4 (the HSV-2 *Bam*HI b'-k' site in U_S) and is compatible with the location previously determined for g92K (21). Individually, all results were consistent with these coordinates, but R12-3 and RD104 did not yield mutually compatible locations. Induction of g92K by R12-3 indicated a right-hand limit at coordinate 0.887 (the HSV-1 *Kpn*I j-h site in U_S); however, RD104, which apparently contains the HSV-2 sequences within these coordinates, failed to induce the glycoprotein. This paradox is discussed later.

Mapping the 92K glycoprotein by SDS-PAGE. To try to understand the anomaly presented by recombinants R12-3 and RD104 and improve on the mapping data for g92K, the polypeptide profiles of 17 HSV-1/HSV-2 intertypic recombinants were studied; 13 of these recombinants were those used in the previous experiment. The presence of g92K and the serotype of gD are presented for all recombinants in Table 2. Figures 5, 6, and 7 show relevant portions of fluorographs of the gels on which [^{14}C]glycosamine-labeled polypeptides induced by B × 6(17-1), B × 1(28-1-1), RD104, RD113, RD213, RE4, RE6, R12-3, and R12-1, the parental 17$^+$ and HG52 strains, and mock-infected cells have been separated. We note that in Fig. 5 the mobility of g92K induced by the three positive recombinants varied from that of g92K induced by HG52, which probably reflects various degrees of processing. Correlation of the data presented in Table 2 with the genome structures of the recombinants (Fig. 4) gives a map position for g92K that is delimited on the left as before by B × 1(28-1-1) and on the right by R12-1, the HSV-2 *Bam*HI c'-d' site in U_S (map coordinate, 0.924). All data were individually consistent with this location, but again R12-3 and RD104 did not yield mutually compatible locations.

Of particular significance were the serotypes of glycoproteins D and E induced by recombinants R12-1 and R12-3. The serotype of pgD could be seen with glucosamine as label (Fig. 5 and 6) and more clearly with mannose as label (Fig. 5). The serotype of gE could be seen with inorganic sulfate as label (Fig. 6) since gE is highly sulfated (15). R12-1 did not induce g92K and did induce gE-2. It also induced a pgD with mobility different from that of either type 1 or type 2, although we note that this mobility difference was not apparent in the mature form of the glycoprotein (gD). R12-3 induced the HSV-2 g92K and HSV-1 glycoproteins D and E. This result demonstrates that g92K and both gD and gE are encoded at least in part by different regions of the genome.

g92K secreted from infected cells. To further characterize g92K, we investigated whether it is secreted from infected cells. A polypeptide which labels heavily with [^{14}C]glucosamine and with the mobility of g92K was secreted from cells infected with 17$^+$ × 11^r, B × 1(28-1-1), and B × 6(17-1) but not from cells infected with RE6, RH6, or RS5 (Fig. 7). Correlation of these data with the genome structures (Fig. 4) gave a map position in the short region (between the HSV-1 *Bam*HI q-m' sites in IR_S and TR_S) with all data consistent, suggesting that the secreted g92K might relate to the intracellular g92K. This suggestion was confirmed and the identity was established by the observation that the secreted g92K reacts with AP1 and LP5 (data not shown).

DISCUSSION

In understanding the genesis of HSV glycoproteins, it has yet to be unambiguously established which of the HSV-1 and HSV-2 glycoproteins are equivalent. The HSV-1 glycoproteins corresponding to HSV-2 gB, gD, and gE are readily identified. Recent work (26, 36, 37) has shown that the HSV-2 equivalent of HSV-1 gC-1 is the glycoprotein identified by Balachandran and co-workers (previously designated gF) (1, 2). To avoid confusion, the glycoprotein identified and originally designated gC-2 by Ruyechan and co-workers (31) has, for the purposes of this discussion, been renamed g124K according to the apparent molecular weight established by this group.

We characterized g92K, an HSV-2 glycoprotein reported by Marsden et al. (21). Four lines of evidence demonstrate that g92K has properties distinct from those assigned to gB,

gC, gD, gE, and g124K. First, the carbohydrate composition and extent of sulfation of g92K differed from those of glycoproteins D or E (Fig. 3). Second, it was not precipitated by monoclonal antibodies against gB, gC-1, gC-2, gE-2 (Fig. 2), and gD (24), whereas two newly produced monoclonal antibodies AP1 and LP5 reacted only with g92K (Fig. 2 and 3). Third, the genomic location of g92K did not coincide with that of gB, gC, or g124K (Fig. 8) (11, 12, 21, 26, 31). Fourth, R12-1 did not induce g92K but did induce gE-2 and an altered glycoprotein D, whereas R12-3 induced the HSV-2 g92K and gD-1 and gE-1 (Fig. 5 and 6), which demonstrates that the g92K is encoded at least in part by a different region of the genome from that encoding gD and gE. Thus, with the exception of g124K, g92K is distinct from the other glycoproteins by at least two of the above lines of evidence. Only the physical map positions of g124K (12, 31) and g92K

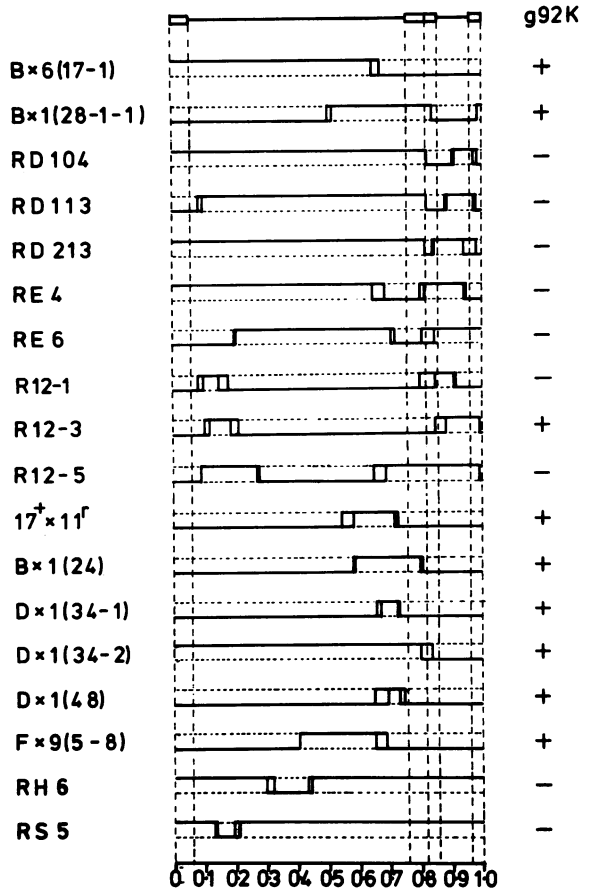

FIG. 4. Summary of the genome structures of the 18 recombinants used in this study. The genome arrangement of HSV DNA is illustrated at the top of the figure, showing the long and short repeat sequences and the long and short unique regions. Vertical dotted lines correspond to the ends of the long and short repeat sequences. 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. The distance between two vertical lines indicates the remaining region of uncertainty for that crossover event. Where the uncertainty is small, the crossover appears as a single vertical line. The units on the bottom are expressed as a fraction of the genome length. The right of the figure shows for each recombinant whether it induces (+) or does not induce (−) g92K.

TABLE 2. Mapping of g92K and gD by SDS-PAGE

Recombinant	Presence of g92K (HSV-2)[a]	gD serotype[b]
B × 6(17-1)	+	2[c]
B × 1(28-1-1)	+	2[c]
RD104	−	1
RD113	−	1
RD213	−	1
RE4	−	1
RE6	−	1
R12-1	−	—[d]
R12-3	+	1
R12-5	−	1
$17^+ \times 11^r$	+	2
B × 1(24)	+	2
D × 1(34-1)	+	2
D × 1(34-2)	+	2
D × 1(48)	+	2
RH6	−	1
RS5	−	1

[a] Serotype determined from [^{14}C]glucosamine-labeled profile.
[b] Serotype determined from both [^{14}C]glucosamine-labeled and [^{3}H]mannose-labeled profiles.
[c] It was not possible to tell the serotype of protein from Fig. 5, but results of other experiments showed these to be HSV-2.
[d] —, The mobility was faster than that of both type 1 and type 2.

distinguish these two glycoproteins. It should be noted that their apparent molecular weights do not distinguish them since these were obtained from different gel systems as discussed below.

The 18 HSV-1/HSV-2 intertypic recombinants used in this study concordantly gave a genomic location for g92K between map coordinates 0.846 and 0.924. However, R12-3 and RD104 gave mutually incompatible locations. Both methods of identification of g92K, by electrophoretic mobility or by type specificity of the antibodies, yielded identical results. Induction of g92K by R12-3 indicated a right-hand limit at coordinate 0.887; however, RD104, which as determined from restriction enzyme analysis (20) contains the HSV-2 sequences within these coordinates, failed to induce the glycoprotein. Without the R12-3 data, the RD104 result indicates a left-hand limit for g92K at the HSV-2 *Bgl*II q-l site (map coordinate, 0.892).

How can the paradox be explained? Failure to observe the predicted induction of a protein by a recombinant has been noted in earlier studies, and possible reasons have been discussed (21). Among these are incorrect protein processing, induction of the protein in subnormal amounts, and incorrect recombinant genome structure due to undetected sequences of DNA from one or another serotype in the recombinant. Figure 8 shows the two possible locations determined for g92K, the map locations of gD-1 obtained by transcript mapping (18, 34, 35) and DNA sequencing (35), the map location of gE-1, obtained by transcript mapping (18), and the right-hand limit of that part of the glycoprotein E gene coding for the difference in mobility between the two serotypes (14). At present we are unable to say which location is correct. Other experimental approaches such as mRNA selection and in vitro translation will have to be employed to obtain the answer.

Why has the 92K glycoprotein not been described by other workers when it appears as such a major band in infected cells labeled with [^{14}C]glucosamine in our experiments (Fig. 1, 2, 3, 5, 6, and 7) (Marsden et al. [21])? Two possible reasons are as follows. First, gels of different compositions

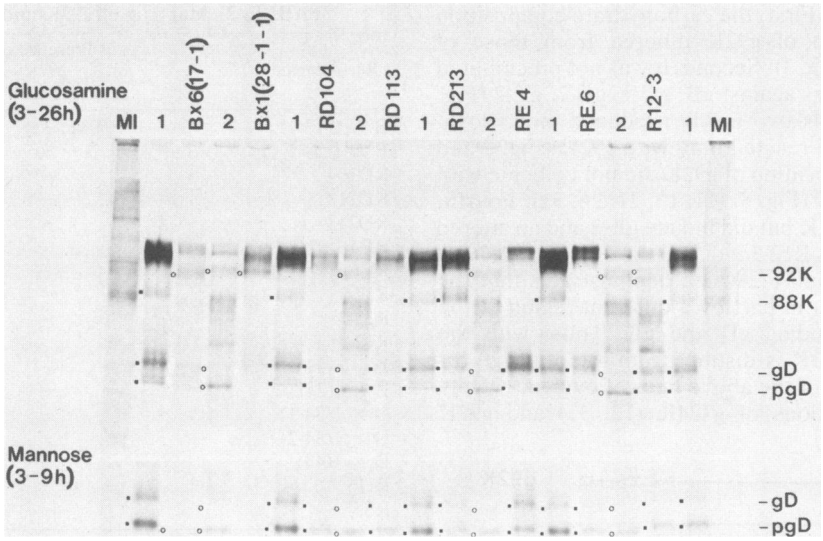

FIG. 5. Mapping the 92K glycoprotein. Fluorograph of polypeptides labeled with [^{14}C]glucosamine (upper panel) and [^{3}H]mannose (lower panel) in mock-infected (MI) cells and in cells infected with B × 6(17-1), B × 1(28-1-1), RD104, RD113, RD213, RE4, RE6, R12-3, and the parental viruses HSV-1 strain 17 *syn*$^{+}$ (lanes 1) and HSV-2 strain HG52 (lanes 2). Polypeptides were separated by electrophoresis in a 5 to 12.5% SDS–polyacrylamide gel. The fluorograph of [^{14}C]glucosamine-labeled polypeptides has been trimmed to show only those polypeptides of molecular weight greater than ca. 40,000, whereas the fluorograph of [^{3}H]mannose-labeled polypeptides has been trimmed to show only those polypeptides of molecular weight between ca. 65,000 and 40,000. Symbols: ■, HSV-1 glycoproteins; ○, HSV-2 glycoproteins.

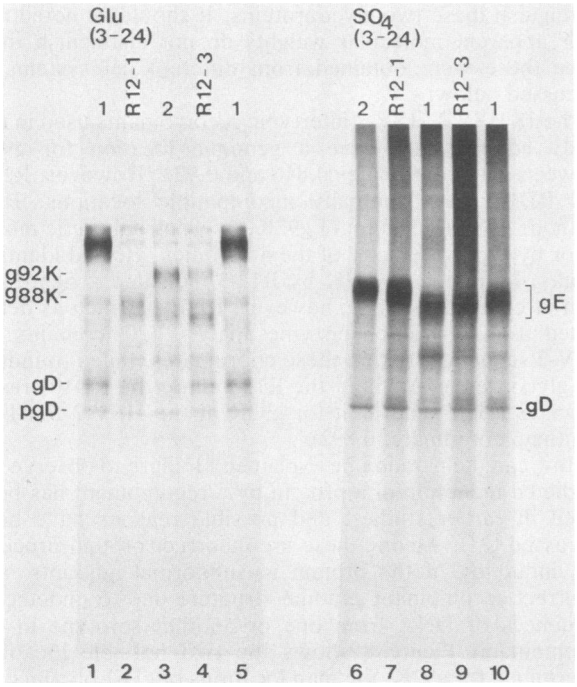

FIG. 6. Presence of g92K and serotype of glycoproteins D and E in recombinants R12-1 and R12-3. Fluorograph of polypeptides labeled with [^{14}C]glucosamine (lanes 1 to 5) or $^{35}SO_4$ (lanes 6 to 10) in cells infected with R12-1, R12-3, and the parental viruses HSV-1 (lanes 1) and HSV-2 (lanes 2). Polypeptides were separated by electrophoresis in a 5 to 12.5% SDS–polyacrylamide gel. Two gels were used; the $^{35}SO_4$-labeled polypeptides migrated a little further than the [^{14}C]glucosamine-labeled ones.

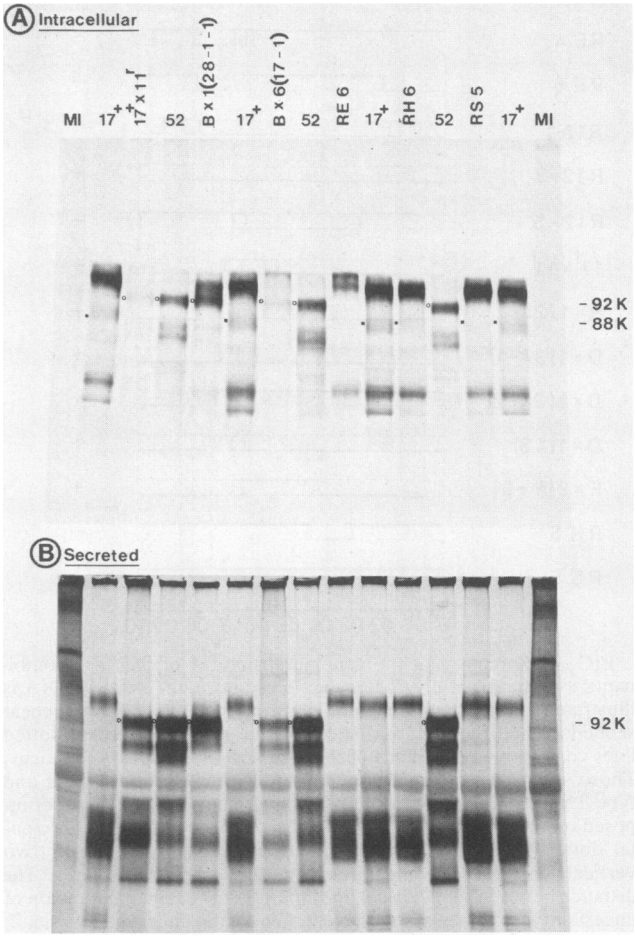

FIG. 7. Mapping the g92K secreted proteins. Autoradiograph of polypeptides induced in cells infected with 17^{+} × 11^{r}, B × 1(28-1-1), B × 6(17-1), RE6, RS5, and the parental viruses 17 *syn*$^{+}$ (HSV-1) (lanes 17^{+}) and HG52 (HSV-2) (lanes 52) and present either intracellularly (panel A) or secreted from the cell (panel B). Cells were labeled with [^{14}C]glycosamine from 2 to 26 h after infection, and polypeptides were separated by electrophoresis in a 5 to 12.5% gradient gel. Symbols: ■, HSV-1 glycoproteins; ○, HSV-2 glycoproteins.

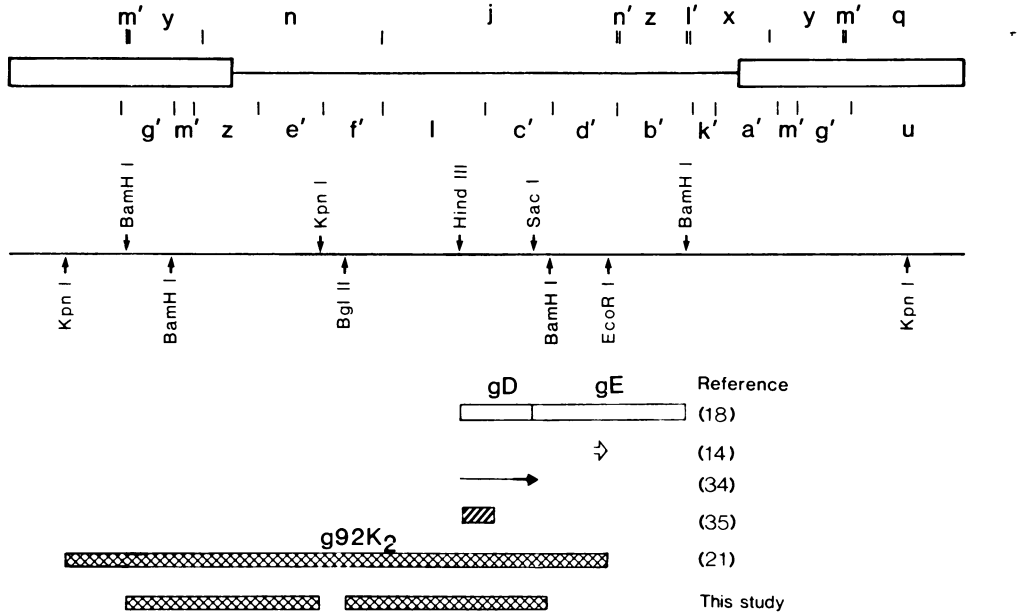

FIG. 8. Map location of the HSV-2-induced g92K. The figure depicts the short region of the genome. The upper section shows the *Bam*HI sites in both HSV-1 DNA (upper letters) and HSV-2 DNA (lower letters). Below this are the restriction enzyme sites which delimit the various glycoproteins: HSV-2 *Kpn*I, a-r; HSV-1 *Bam*HI, q-m'; HSV-2 *Bam*HI, g'-m'; HSV-1 *Kpn*I, j-h; HSV-2 *Bgl*II, q-l; HSV-1 *Hin*dIII, g-n; the middle *Sac*I site in HSV-1 *Bam*HI, j; HSV-2 *Eco*RI, n-o; HSV-1 *Bam*HI, z-x; HSV-2 *Kpn*I, a-r. The genomic location of gD-1 and gE-1 obtained by mRNA selection and in vitro translation (18) are shown. The open arrow denotes the right-hand limit of that part of the glycoprotein E gene coding for the difference in mobility between the two serotypes (14). The long solid arrow shows the direction of transcription and the location of the mRNA for gD-1 (34), and the hatched region shows the polypeptide coding sequence (35). The cross-hatched region shows the location obtained previously for g92K (21) and the two possible locations deduced from the data presented here.

are known to have different resolving properties for particular glycoproteins. Thus, in the two gel systems used in these studies, g92K migrates faster than gB. However, in 9% gels cross-linked with *N,N'*-diallyltartardiamide, a system commonly employed by others, g92K migrates relatively more slowly, close to gB, and is masked by the glycoproteins in this region of the gel (data not shown). Second, the host cell type used to culture the virus may well be important since the apparent molecular weights of HSV glycoproteins are known to be influenced by the host cell. This possibility has not been investigated.

Several investigators have reported glycoproteins to be secreted from HSV-infected cells (6, 15, 16, 23, 30). Here we demonstrated that g92K secreted from infected cells corresponds to the intracellular g92K (Fig. 7). A protein of this molecular weight was first shown to be secreted from HSV-2-infected cells by Randall et al. (30), who designated it ICRP1. Our basis for suggesting identity between ICRP1 and g92K is the similarity of the electrophoretic profiles of secreted proteins as discussed elsewhere (15).

No HSV-1 glycoprotein equivalent to g92K has been rigorously identified. We now propose that either it has no equivalent or, if it has, then g88K (Fig. 5, 6, and 7) is a likely candidate. This is based on the data presented here, which consistently show that no recombinant induces both g88K and g92K. These two proteins are, like most equivalent HSV-1 and HSV-2 proteins, of similar electrophoretic mobility. But it is relevant that RD104 and RE6 induce neither g88K nor g92K in detectable amounts (Fig. 5 and 7). If they are equivalent, then this observation suggests that, like in the case of gC-1 (13, 32), synthesis of normal amounts of g92K or g88K is not essential for a productive infection of cells in tissue culture. If they are not related then it will be important to establish whether equivalents to both g92K and g88K exist and what they are. Further progress in understanding this question will be helped by production of monospecific antibodies against g88K.

Since submission of this manuscript we learned that the previous mapping of g124K (31) is now considered to be incorrect and that more recent studies (B. Roizman, B. Norrild, C. Chan, and L. Pereira, submitted for publication) place it in U_S, overlapping with the position of g92K. g124K was redefined as the glycoprotein reactive with the monoclonal antibody H966 and was newly designated gG. Besides having compatible molecular weights (see earlier discussion) and overlapping map positions, gG and g92K are similar in that no equivalent HSV-1 glycoprotein has yet been identified. These three common properties led the authors to suggest that gG and g92K might be one and the same protein. However, this suggestion will have to be rigorously tested by comparing the reactivities of the monoclonal antibodies defining the two glycoproteins.

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