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Isolation, Characterization, and Physical Mapping of a Pseudorabies Virus Mutant Containing Antigenically Altered gp50

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A pseudorabies virus variant (*mar*197-1) containing a mutation in a viral glycoprotein with a molecular weight of 50,000 (gp50) was isolated by selecting for resistance to a neutralizing monoclonal antibody (MCA50-1) directed against gp50. This mutant was completely resistant to neutralization with MCA50-1 in the presence or absence of complement, and was therefore defined as a *mar* (monoclonal-antibody-resistant) mutant. The mutation did not affect neutralization with polyvalent immune serum. The *mar*197-1 mutant synthesized and processed gp50 normally, but the mutation prevented the binding and immunoprecipitation of gp50 by MCA50-1. Thus, the mutation was within the structural portion of the gp50 gene affecting the epitope of the monoclonal antibody. The mutation was mapped by marker rescue with cloned pseudorabies restriction enzyme fragments to the short unique region of the pseudorabies genome between 0.813 and 0.832 map units. This is equivalent to a 2.1-kilobase-pair region.

Pseudorabies virus (PRV) contains a double-stranded DNA genome with a molecular weight of ca. 90×10^6 (32). The genome can be divided into long and short unique regions, with the short unique region surrounded by inverted repeat sequences (4). Like other alpha herpesviruses, PRV has a short replicative cycle in cell culture, resulting in mass destruction of susceptible cells (31). In swine (its natural host), PRV can cause death in pigs of all ages and abortion of fetuses (19), thus making it an economically important virus.

Since glycoproteins are present on the surface of a herpesvirus envelope, they are the principal target antigens involved in virus neutralization (9, 15, 42). The envelope of herpes simplex virus type 1 (HSV-1) contains at least four antigenically distinct glycoproteins, designated gB, gC, gD, and gE (2, 20, 37). Glycoproteins gB, gC, and gD appear to be the principal immunogens involved in inducing both the humoral and cellular host immune responses (16, 25, 27, 42).

PRV also has been reported to have four glycoproteins with molecular weights estimated to be 100,000 (100k) to 120k, 72k to 82k, 60k to 65k, and 45k to 50k (3, 23; L. K. Wathen, K. B. Platt, M. W. Wathen, R. A. Van Deusen, and C. A. Whetstone, manuscript in preparation). A monoclonal antibody directed against the glycoprotein with a molecular weight of 50,000 (gp50) is a strong neutralizer of PRV with or without the aid of complement (Wathen et al., in preparation). Furthermore, polyvalent immune serum is highly reactive against gp50. It therefore appears that gp50 is one of the important PRV immunogens.

Neutralizing monoclonal antibodies directed against HSV-1 glycoproteins have been used to select viral antigenic variants which are resistant to neutralization (21). These antigenic variants usually express the mutated glycoprotein but do not bind the selecting monoclonal antibody due to an alteration in the epitope against which the monoclonal antibody is directed (21). Such mutants are distinct from the normal conditional lethal mutants and have been designated *mar* (monoclonal-antibody-resistant) mutants (21).

We report here on the isolation and analysis of an antigenic variant resistant to a neutralizing monoclonal antibody directed against the PRV gp50. We used this *mar* mutant to map the gp50 gene to an area within the short unique region of the PRV genome. The similarities between this PRV glycoprotein and glycoprotein gD of HSV-1 are discussed.

MATERIALS AND METHODS

Virus and cell culture. Plaque-purified PRV strain Ind-FH (34) was routinely passed at a multiplicity of infection of 0.01 PFU/ml in porcine MVPK-1 cells (41). Cells were propagated in Eagle minimal essential medium (MEM) containing 10% fetal calf serum (FCS). Mutagenized stocks of PRV were prepared by adding 5-bromo-2'-deoxyuridine (BUdR; 10 μg/ml in MEM–10% FCS) at 1 h postinfection (p.i.). BUdR remained in the medium until the extracellular virus was harvested.

Production of monoclonal antibody stocks. Production and characterization of the hybridoma cell line MCA50-1, as well as the preparation of ascites fluid used in this paper, will be described elsewhere (Wathen et al., in preparation). Clarified ascites fluid from mice injected intraperitoneally with MCA50-1 hybridoma cells was used without further purification.

Virus neutralization assay. Titers of monoclonal ascites fluid or porcine immune serum to PRV were determined by serial twofold dilutions in 96-well tissue culture plates. The assays were performed by mixing 50 μl of serially diluted antibody which had been incubated at 56°C for 30 min with ca. 100 PFU (50 μl) of virus. Complement-mediated neutralization was assayed by the addition of 0.5% normal rabbit serum. After a 3-h incubation at 37°C, 10^4 MVPK-1 cells were added to each well, and the assay was incubated at 37°C for 48 h. Antibody titers were expressed as the reciprocal of the highest dilution which reduced the viral cytopathic effect (CPE) by 50% relative to unneutralized controls.

Isolation of *mar* mutants. Antigenic variants of PRV were selected on the basis of their resistance to neutralization with the monoclonal antibody MCA50-1. Approximately 2×10^5 PFU of mutagenized virus was incubated with 0.25 ml of MCA50-1 ascites fluid for 3 h at 37°C. Surviving virus was allowed to adsorb to MVPK-1 cells at 37°C for 1 h. The cell monolayer was then washed twice and overlaid with 0.8% agar containing MEM–5% FCS. At 48 h p.i., plaques were

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picked and dispensed into 24-well tissue culture plates containing confluent monolayers of MVPK-1 cells. When the infection in these cells reached 100% CPE, the progeny virus was tested for resistance to MCA50-1 by using the neutralization assay described above. Suspected *mar* mutants were plaque purified twice and retested for resistance to neutralization. One *mar* mutant (*mar*197-1) was chosen for further experimentation.

Radiolabeling of cells. MVPK-1 cells (5×10^6) were infected with PRV at a multiplicity of infection of 50 PFU per cell. After a 1-h adsorption period, the cells were washed twice, and fresh MEM–10% FCS was added at 37°C. At 16 h p.i., 2 μCi of [^{14}C]glucosamine per ml (54 mCi/mmol; Amersham Corp., Arlington Heights, Ill.) was added, and the cells were incubated at 37°C. The cells were harvested at 20 h p.i. by washing twice and then scraping into phosphate-buffered saline (PBS) (pH 7.4). The cells were pelleted and resuspended in 0.5 ml of extraction buffer (1% Nonidet P-40, 0.1 mM phenylmethylsulfonyl fluoride in PBS, pH 8.0). After a brief sonication, the disrupted cells were solubilized for 15 min at 4°C. Extracts were clarified by centrifugation at 12,000 × *g* for 15 min.

Immunoprecipitations. Immunoprecipitations were carried out as previously described (21). Briefly, radiolabeled cell extracts (100 μl) were incubated with 5 μl of ascites fluid or 20 μl of porcine immune serum overnight at 4°C. Antigen-antibody complexes were harvested by the addition of 50 μl of protein A-Sepharose CL-4B beads (40% [vol/vol] in extraction buffer; Pharmacia Fine Chemicals, Piscataway, N.J.). The beads that were added to monoclonal antibody immunoprecipitates were presensitized with rabbit anti-mouse immunoglobulin (Cappel Laboratories, Cochranville, Pa.). After incubating for 1 h at 4°C, the beads were washed six times with extraction buffer and resuspended in 25 μl of 2× electrophoresis sample buffer (2% sodium dodecyl sulfate, 5% 2-mercaptoethanol, 10% sucrose, 2 mg of bromphenol blue per ml, 10 mM Tris-hydrochloride, pH 8.0).

Polyacrylamide gel electrophoresis. Immunoprecipitates and total cell extracts (5 μl in 2× electrophoresis sample buffer) were boiled for 3 min before sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Sepharose beads from the immunoprecipitations were removed by centrifugation. The polypeptides were then fractionated in a resolving gel containing 10% acrylamide and 1.3 mg of bisacrylamide (Bio-Rad Laboratories, Richmond, Calif.) per ml. The stacking gel contained 5% acrylamide and 1.3 mg of bisacrylamide per ml. Electrophoresis was carried out at 75 V through the stacking gel and 150 V through the resolving gel by the method of Laemmli (24). Molecular weight standards (Bio-Rad Laboratories) were coelectrophoresed in each gel. After electrophoresis, gels were stained, destained, and dried as previously described (38). Dried gels were exposed to Kodak X-Omat AR film at room temperature.

Purification of viral DNA. Viral DNA used for cloning the PRV genome was purified from cytoplasmic virions by a modification of the sodium iodide-ethidium bromide gradient centrifugation procedure of Walboomers and Schegget (43) as previously described (28). Viral DNA used for marker rescue experiments was purified from PRV nucleocapsids as previously described (40).

Cloning DNA restriction enzyme fragments of PRV. *Bam*HI restriction enzyme fragments of PRV DNA were inserted into the plasmid pACYC184 (8) by using standard cloning procedures. Subcloning of *Bam*HI fragment H with *Sal*I was carried out in pBR325 (7). The plasmids pY165D, pY104H, and pY109K containing the PRV *Bam*HI fragments DKN, H, and K, respectively, that were cloned into pBR322 were kindly provided by Prem S. Paul.

Ligated recombinant plasmid DNA was transformed into *Escherichia coli* HB101 by the calcium shock method of Cohen et al. (11). Cells were plated onto LB agar plates containing 25 μg of chloramphenicol per ml (pACYC184) or 200 μg of ampicillin per ml (pBR325).

Recombinant plasmid-containing colonies were initially screened by insertional inactivation of an antibiotic resistance marker. Plasmid DNA was purified by using a sodium hydroxide-sodium dodecyl sulfate extraction procedure (6), followed by cesium chloride-ethidium bromide equilibrium centrifugation (18). The identity of the viral insert was preliminarily determined by digestion of recombinant plasmid DNA with various restriction enzymes (New England Biolabs, Beverly, Mass.) and electrophoresis in agarose gels by the method of Bachi and Arber (1). The identity of the DNA insert was verified by hybridizing ^{32}P-labeled plasmid DNA to *Kpn*I-digested PRV DNA fixed to nitrocellulose filters by the method of Southern (36). Plasmid DNA was radiolabeled in vitro by the nick translation method of Rigby et al. (30).

Excision of DNA fragments from agarose gels. DNA restriction enzyme fragments were recovered from horizontal agarose gels by band interception onto NA-45 DEAE membranes (Schleicher and Schuell, Keene, N.H.) by using a modification of the method of Dretzen et al. (13). The NA-45 membranes were washed for 10 min in 10 mM EDTA (pH 7.5) and 5 min in 0.5 M NaOH and then were rinsed several times in deionized water. The activated membranes were then placed in slits in the agarose gel directly below the DNA bands of interest, and the DNA was electrophoresed onto the NA-45 membranes. After residual agarose was rinsed from the membranes with NET buffer (0.15 M NaCl, 0.1 mM EDTA, 20 mM Tris-hydrochloride, pH 8.0), the DNA was eluted from the membranes by incubation in high salt (1.0 M NaCl) NET buffer at 56°C for 45 min. The DNA was then extracted three times with an equal volume of water-saturated *n*-butanol and was ethanol precipitated.

Marker rescue of *mar* mutant with recombinant plasmids. The marker rescue technique used was essentially that of Stow et al. (39). Briefly, MVPK-1 cells at ca. 90% confluency were cotransfected with DNA from intact PRV (*mar*197-1) mutant virus and PRV recombinant plasmids by the calcium phosphate infectivity technique of Graham and van der Eb (17). Plasmid DNA had been digested with *Bam*HI or *Bam*HI-*Sal*I, phenol extracted three times, chloroform extracted twice, and ethanol precipitated. At 4 h p.i., the cells were subjected to a 3-min polyethylene glycol-sucrose shock as previously described (35). Extracellular virus was harvested when the infection reached 100% CPE (3 to 4 days p.i.).

Assay for marker rescue. Recombinant virus which had regained a wild-type gp50 was detected with an in situ enzyme immunoassay described by Holland et al. (22). Briefly, progeny virus from the transfections were plated onto 60-mm petri dishes containing a monolayer of MVPK-1 cells at 100 to 1,000 PFU per petri dish. After a 1-h adsorption period, the cells were washed and overlaid with 0.8% agarose containing MEM–5% FCS. At 2 days p.i., the overlay was removed, and the cells were fixed with glutaraldehyde (0.25% in PBS, pH 7.4) for 5 min. The cells were washed three times with PBS (pH 7.4) containing 3% bovine serum albumin (BSA) (PBS-BSA) and then incubated for 2 h at room temperature with a 1:1,000 dilution of MCA50-1 ascites fluid in PBS-BSA. After unbound monoclonal anti-

body was removed by three washes with PBS-BSA, the cells were incubated for 2 h at room temperature with 2 ml of horseradish peroxidase-conjugated goat anti-mouse immunoglobulin (Cappel Laboratories) diluted 1:250 in PBS-BSA. After this incubation, the cells were washed three times with PBS-BSA and incubated in 2 ml of substrate containing 4-chloro-1-naphthol and H_2O_2. Plaques with a wild-type morphology stained black in 10 to 20 min, whereas nonrescued virus remained unstained.

RESULTS

Characterization of PRV (*mar*197-1) mutant. Antigenic variants of PRV (Ind-FH) containing a mutation in gp50 were selected on the basis of resistance to a neutralizing monoclonal antibody (MCA50-1) directed against gp50. Approximately 4% of the BUdR-treated stock virus survived neutralization with MCA50-1, and of the survivors approximately 1% were mutants. Although an exact mutation rate for the gp50 gene could not be determined because of the low number of mutants isolated, other experiments with BUdR-treated PRV yielded a mutation frequency for the gp50 gene of 3×10^{-5} (unpublished data).

The mutant *mar*197-1 was chosen for further experimentation because of its complete resistance to MCA50-1. This antigenic variant was resistant to MCA50-1 in the presence or absence of complement at the lowest dilution tested (Table 1). Neutralization with immune serum from a PRV-infected pig was unaffected by the mutation (Table 1).

The inability of MCA50-1 to bind the mutated gp50 was demonstrated by immunoprecipitation of [^{14}C]glucosamine-labeled infected cell extracts (Fig. 1). Comparison of labeled total cell extract from mutant (Fig. 1, lane 1) and wild-type (Fig. 1, lane 2) infected cells indicated that gp50 was being synthesized in the proper amounts and processed to the correct size by *mar*197-1. This was substantiated by immunoprecipitation of the cell extracts with porcine immune serum (Fig. 1, lanes 3 and 4). However, MCA50-1, which readily immunoprecipitated wild-type gp50 (Fig. 1, lane 6), did not immunoprecipitate mutant gp50 in detectable amounts (Fig. 1, lane 5).

Cloning PRV *Bam*HI restriction enzyme fragments. To obtain adequate amounts of uncontaminated PRV restriction enzyme fragments for use as probes in marker rescue experiments, PRV DNA was cleaved with *Bam*HI and cloned into the plasmid vectors pACYC184 or pBR322 as described above. The two plasmid vectors can be differentiated on agarose gels by the slower electrophoretic migration of pBR322 (Fig. 2, plasmids DKN, H, and K). Two of the

TABLE 1. Neutralization of *mar* mutant and wild-type PRV[a]

Antibody	Complement	Neutralization titer[b] for:	
		*mar*197-1	Ind-FH
MCA50-1[c]	−	<200[d]	205,000
MCA50-1	+	<200	640,000
Immune serum[e]	−	51,000	51,000
Immune serum	+	102,000	102,000

[a] The two PRV isolates tested were the mutant *mar*197-1, which has antigenically altered gp50, and the wild-type strain Ind-FH.

[b] The neutralization titer is expressed as the reciprocal of the highest dilution of antibody which reduced the viral CPE by 50%.

[c] Clarified ascites fluid of monoclonal antibody directed against gp50.

[d] Dilutions below 1:200 were not tested because of toxicity to cells from the ascites fluid.

[e] Multivalent serum from a pig which had been naturally infected with a field strain of PRV.

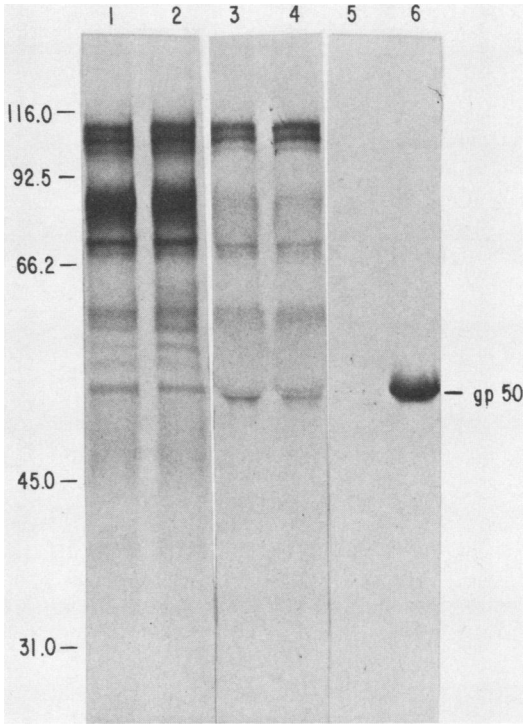

FIG. 1. Autoradiograph of 10% sodium dodecyl sulfate-polyacrylamide gel containing [^{14}C]glucosamine-labeled glycoproteins. Clarified cell extracts from the mutant *mar*197-1 (lane 1) and wild-type Ind-FH (lane 2) were either directly compared or immunoprecipitated with porcine immune serum (lane 3, *mar*197-1; lane 4, Ind-FH) or monoclonal antibody MCA50-1 (lane 5, *mar*197-1; lane 6, Ind-FH). The migration of molecular weight markers ($\times 10^3$) and gp50 are indicated.

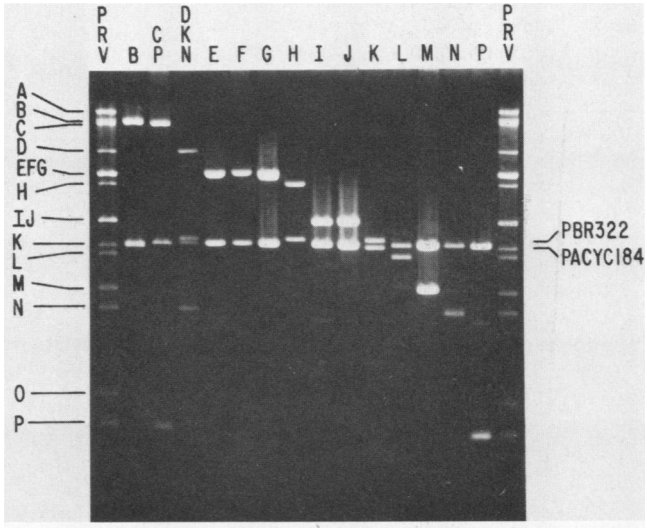

FIG. 2. Ethidium bromide-stained agarose gel containing PRV recombinant clones. PRV DNA was digested with *Bam*HI and electrophoresed in the outer lanes as a reference. The letter denoting each PRV *Bam*HI restriction enzyme fragment is shown on the left. *Bam*HI-digested recombinant clones containing PRV *Bam*HI restriction enzyme fragments were arranged in the gel according to insert size. The PRV insert(s) in each clone is indicated above its lane. The migration of the two cloning vectors pBR322 and pACYC184 is marked on the right.

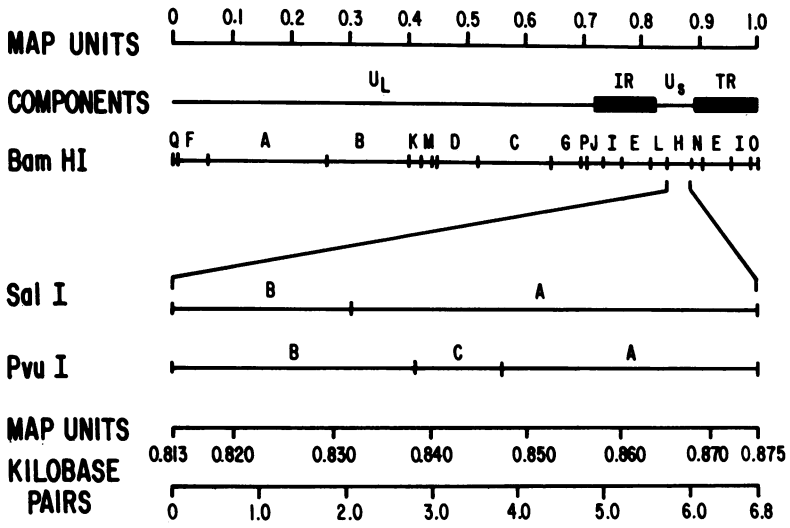

FIG. 3. Physical map of the *Bam*HI cleavage sites for PRV (Ind-FH) and of the *Sal*I and *Pvu*I cleavage sites for *Bam*HI-H. The *Bam*HI restriction enzyme map was originally described for the Shope strain of PRV (14) and was verified to be correct for strain Ind-FH by Southern blot hybridization (unpublished data). *Bam*HI-H lies within the short unique section of the PRV genome between 0.813 and 0.875 map units and is 6.8 kilobase pairs long. Restriction enzyme fragments within the *Bam*HI-H region were alphabetically labeled according to size for each enzyme.

recombinant plasmids (CP and DKN) contained multiple PRV restriction enzyme fragments. However, the contribution of each fragment in these clones toward the ability of the clone to rescue a mutation could be determined by comparison with the single fragment clones K, N, and P (Fig. 2). As expected, the end fragments O and Q (not detectable in Fig. 2) were not cloned. Since the sequences represented by *Bam*HI-O are within the terminal repeat region (Fig. 3), these sequences are also present in the portion of the internal repeat represented by clone J (Fig. 2 and 3). The PRV sequences represented by restriction enzyme *Bam*HI fragments A and Q were not present in this library of clones.

Marker rescue of *mar*197-1 with PRV *Bam*HI clones. The genomic map location of the *mar* mutation in the gp50 gene of PRV (*mar*197-1) was determined by marker rescue with the PRV recombinant clones described above. Rescued virus was detected by using an in situ enzyme immunoassay described above. This assay is based on the fact that only wild-type plaques derived from rescued virus will bind the monoclonal antibody MCA50-1. Such plaques stain black under the assay conditions, whereas mutant plaques remain unstained. A petri dish containing virus from a transfection in which a fraction of the *mar*197-1 virus was rescued by recombination with a PRV clone is shown in Fig. 4.

The results of cotransfections with DNA from *mar*197-1 and PRV clones containing *Bam*HI restriction enzyme fragments are presented in Table 2. Only *Bam*HI-H was able to rescue the mutation in the gp50 gene. This fragment is contained within the short unique region of the PRV genome between 0.813 and 0.875 map units (Fig. 3). Background levels of marker rescue with the other *Bam*HI clones or reversion of *mar*197-1 were below detectability (Table 2).

Marker rescue of *mar*197-1 with restriction enzyme fragments from the *Bam*HI-H region. A map of the *Bam*HI-H region is shown in Fig. 3. This map was derived by single and double restriction enzyme digestions of the *Bam*HI-H clone with *Bam*HI, *Sal*I, and *Pvu*I. The *Sal*I fragments were subcloned by digesting the *Bam*HI-H clone with *Bam*HI-*Sal*I and inserting the resulting fragments into pBR325 which had been digested with *Bam*HI-*Sal*I. This double digestion removed the viral DNA from the original pBR322 plasmid vector. The *Pvu*I viral fragments were isolated from agarose gels after electrophoresis of *Pvu*I-digested *Bam*HI-H clone as described above.

The results of marker rescue experiments with the *Sal*I subclones or *Pvu*I fragments from the *Bam*HI-H region are presented in Table 3. These results indicate that the mutation in the gp50 gene of *mar*197-1 is located at the left end of *Bam*HI-H between 0.813 and 0.832 map units (Fig. 3, *Sal*I-B).

DISCUSSION

We used a neutralizing monoclonal antibody (MCA50-1) directed against PRV gp50 to select an antigenic variant (*mar*197-1) which is resistant to neutralization with MCA50-

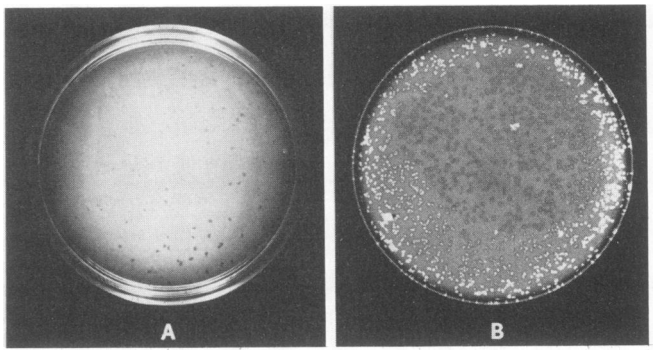

FIG. 4. Comparison of in situ enzyme immunoassay and crystal violet staining. Progeny virus from transfections with mutant *mar*197-1 DNA and cloned fragments of PRV were assayed for marker rescue as described in the text. (A) Wild-type plaques from rescued virus stained black under the immunoassay conditions. (B) After the number of wild-type plaques were counted, the total number of plaques were counted by staining with 0.1% crystal violet.

1. This antigenic variant was completely resistant to neutralization in the presence or absence of complement. The *mar*197-1 gp50 could not be differentiated from wild-type gp50 by its size or abundance in infected cells, indicating that the mutation did not result in improper processing and was not in the control region of the gp50 gene. The mutation in *mar*197-1 did prevent the immunoprecipitation of gp50 by MCA50-1. These results prove that the mutation is in the structural portion of gp50 and that the resistance to neutralization is due to the inability of MCA50-1 to bind the antigenically altered gp50. Since BUdR is a base analog mutagen, the mutation in *mar*197-1 is probably a point mutation. So the inability of MCA50-1 to bind the antigenically altered gp50 is probably due to the change of one amino acid within the otherwise complementary epitope.

Because of its inability to bind MCA50-1, *mar*197-1 could be used in marker rescue experiments to map the gp50 gene. Rescued virus was detected by using an in situ enzyme immunoassay which stained black that virus capable of binding MCA50-1. Using this procedure, we mapped the mutation within the gp50 gene to the short unique region of the PRV genome between 0.813 and 0.832 map units. This is equivalent to a 2.1-kilobase-pair region. The coordinates provided above only demarcate the location of a mutation within the gp50 gene. The mRNA coding for this glycoprotein may extend beyond these limits.

The importance of glycoproteins to the host immune response elicited against a herpesvirus infection has been well documented (15, 25, 27, 42). The glycoprotein gD of HSV-1 has been shown to be a type-common antigen capable of inducing a host immune response which can protect against both HSV-1 and HSV-2 (10). For these reasons, the effort to produce a recombinant HSV vaccine by expression of a viral antigen in *E. coli* has centered on gD (44, 45). The PRV gp50 has several similarities to HSV-1 gD. The estimated molecular weight of both glycoproteins is ca. 50k (3, 29; Wathen et al., in preparation), and they both map in the short unique region of their respective genomes (26, 33, 44). Furthermore, some monoclonal antibodies directed against gD and gp50 can neutralize their respective viruses without the aid of complement (21; Wathen et al., in preparation). Despite these similarities, no DNA sequence homology between HSV and PRV has been detected in the regions coding for these proteins (5, 12). Further studies will be required to determine if HSV-1 gD and PRV gp50 are functionally related.

TABLE 2. Marker rescue of *mar*197-1 with *Bam*HI restriction enzyme fragments[a]

Restriction enzyme fragment	Total no. of plaques	No. of black plaques[b]	% Rescue
	>2,000	0	<0.05
B	>2,000	0	<0.05
CP[c]	>2,000	0	<0.05
DKN[c]	652	0	<0.2
E	>2,000	0	<0.05
F	160	0	<0.6
G	458	0	<0.2
H (expt 1)	1,037	280	27
H (expt 2)	6,754	388	5.7
I	>2,000	0	<0.05
J	>2,000	0	<0.05
K	268	0	<0.3
L	>2,000	0	<0.05
M	1,925	0	<0.05
N	>2,000	0	<0.05
P	>2,000	0	<0.05

[a] Intact DNA (0.5 μg) from the PRV mutant *mar*197-1 was cotransfected with 5 μg of a recombinant plasmid containing a PRV *Bam*HI restriction enzyme fragment into MVPK-1 cells as described in the text. Extracellular virus was harvested when the CPE was generalized. This virus was plated onto 60-mm petri dishes, and the resultant plaques were assayed for rescue of mutated gp50 by an in situ enzyme immunoassay.

[b] A black plaque indicates that the virus was able to bind monoclonal antibody MCA50-1 and therefore had regained wild-type gp50.

[c] The clone contained multiple PRV *Bam*HI restriction enzyme fragments.

TABLE 3. Marker rescue of *mar*197-1 with fragments from the *Bam*HI-H region[a]

Restriction enzyme fragment	Total no. of plaques	No. of black plaques[b]	% Rescue
*Pvu*I-A[c]	1,669	0	<0.06
*Pvu*I-B	6,732	8	0.1
*Pvu*I-C	1,969	0	<0.05
*Sal*I-A (expt 1)[d]	3,320	0	<0.03
*Sal*I-A (expt 2)	14,421	0	<0.007
*Sal*I-B (expt 1)	1,836	11	0.6
*Sal*I-B (expt 2)	9,218	28	0.3

[a] Marker rescue of the PRV mutant *mar*197-1 containing antigenically altered gp50 was carried out as described in Table 2, footnote *a*, and in the text.

[b] A black plaque indicates that the virus was able to bind monoclonal antibody MCA50-1 and therefore had regained wild-type gp50.

[c] PRV *Pvu*I fragments from the *Bam*HI-H clone were recovered from agarose gels by band interception onto NA-45 DEAE membranes. Approximately 5-μg equivalents of each fragment were used for marker rescue.

[d] *Sal*I subclones of the *Bam*HI-H clone were obtained by digestion with *Bam*HI-*Sal*I and insertion into pBR325 which had been digested with *Bam*HI-*Sal*I. Before transfection, 5 μg of each clone was digested with *Bam*HI and *Sal*I to release the viral DNA from the plasmid.

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LITERATURE CITED

1. **Bachi, B., and W. Arber.** 1977. Physical mapping of BglII, BamHI, EcoRI, HindIII, and PstI restriction fragments of bacteriophage P1 DNA. Mol. Gen. Genet. **153**:311–324.
2. **Baucke, R. B., and P. G. Spear.** 1979. Membrane proteins specified by herpes simplex viruses. V. Identification of an Fc-binding glycoprotein. J. Virol. **32**:779–789.
3. **Ben-Porat, T., and A. S. Kaplan.** 1970. Synthesis of proteins in cells infected with herpesvirus. V. Viral glycoproteins. Virology **41**:265–273.
4. **Ben-Porat, T., F. J. Rixon, and M. L. Blankenship.** 1979. Analysis of the structure of the genome of pseudorabies virus. Virology **95**:285–294.
5. **Ben-Porat, T., R. A. Veach, and S. Ihara.** 1983. Localization of the regions of homology between the genomes of herpes simplex virus type 1 and pseudorabies virus. Virology **127**:194–204.
6. **Berman, M. L., L. W. Enquist, and T. J. Silhavy.** 1981. Advanced bacterial genetics, p. 84–85. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
7. **Bolivar, F.** 1978. Construction and characterization of new cloning vehicles. III. Derivatives of plasmid pBR322 carrying unique EcoRI sites for selection of EcoRI generated recombinant DNA molecules. Gene **4**:121–136.
8. **Chang, A. C. Y., and S. N. Cohen.** 1978. Construction and characterization of amplifiable multicopy DNA cloning vehicles derived from the P15A cryptic miniplasmid. J. Bacteriol. **134**:1141–1156.
9. **Choppin, P. W., and A. Scheid.** 1980. The role of viral glycoproteins in adsorption, penetration, and pathogenicity of viruses.

Rev. Infect. Dis. **2**:40–61.
10. **Cohen, G. H., M. Katze, C. Hydrean-Stern, and R. J. Eisenberg.** 1978. Type-common CP-1 antigen of herpes simplex virus is associated with a 59,000-molecular-weight envelope glycoprotein. J. Virol. **27**:172–181.
11. **Cohen, S. N., A. C. Y. Chang, and L. Hsu.** 1973. Construction of biologically functional bacterial plasmids in vitro. Proc. Natl. Acad. Sci. U.S.A. **70**:3240–3244.
12. **Davidson, A. J., and N. M. Wilkie.** 1983. Location and orientation of homologous sequences in the genomes of five herpesviruses. J. Gen. Virol. **64**:1927–1942.
13. **Dretzen, G., M. Bellard, P. Sassone-Corsi, and P. Chambon.** 1981. A reliable method for the recovery of DNA fragments from agarose and acrylamide gels. Anal. Biochem. **112**:295–298.
14. **Feldman, L., F. J. Rixon, J. H. Jean, T. Ben-Porat, and A. S. Kaplan.** 1979. Transcription of the genome of pseudorabies virus (a herpesvirus) is strictly controlled. Virology **97**:316–327.
15. **Glorioso, J. C., and J. W. Smith.** 1977. Immune interaction with cells infected with herpes simplex virus: antibodies to radioiodinated surface antigens. J. Immunol. **118**:114–121.
16. **Glorioso, J. C., M. Levine, T. C. Holland, and M. S. Szczesiul.** 1980. Mutant analysis of herpes simplex virus-induced cell surface antigens: resistance to complement-mediated immune cytolysis. J. Virol. **35**:672–681.
17. **Graham, E. L., and A. J. van der Eb.** 1973. A new technique for the assay of infectivity of human adenovirus 5 DNA. Virology **52**:456–467.
18. **Guerry, P., D. J. LeBlanc, and S. Falkow.** 1973. General method for the isolation of plasmid deoxyribonucleic acid. J. Bacteriol. **116**:1064–1066.
19. **Gustafson, D. P.** 1975. Pseudorabies virus, p. 391–410. *In* H. W. Dunne and A. D. Leman (ed.), Diseases of swine. The Iowa State University Press, Ames.
20. **Heine, J. W., R. W. Honess, E. Cassai, and B. Roizman.** 1974. Proteins specified by herpes simplex virus. XII. The virion polypeptides of type 1 strains. J. Virol. **14**:640–651.
21. **Holland, T. C., S. D. Marlin, M. Levine, and J. Glorioso.** 1983. Antigenic variants of herpes simplex virus selected with glycoprotein-specific monoclonal antibodies. J. Virol. **45**:672–682.
22. **Holland, T. C., R. M. Sandri-Goldin, L. E. Holland, S. D. Marlin, M. Levine, and J. C. Glorioso.** 1983. Physical mapping of the mutation in an antigenic variant of herpes simplex virus type 1 by use of an immunoreactive plaque assay. J. Virol. **46**:649–652.
23. **Killington, R. A., J. Yeo, R. W. Honess, D. H. Watson, B. E. Duncan, I. W. Halliburton, and J. Mumford.** 1977. Comparative analyses of the proteins and antigens of five herpesviruses. J. Gen. Virol. **37**:297–310.
24. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
25. **Lawman, M. J. P., R. J. Courtney, R. Eberle, P. A. Schaffer, M. K. O'Hara, and B. T. Rouse.** 1980. Cell-mediated immunity to herpes simplex virus: specificity of cytotoxic T cells. Infect. Immun. **30**:451–461.
26. **Lee, G. T.-Y., M. F. Para, and P. G. Spear.** 1982. Location of the structural genes for glycoproteins gD and gE and for other polypeptides in the S component of herpes simplex virus type 1 DNA. J. Virol. **43**:41–49.
27. **Norrild, B., S. L. Shore, and A. J. Nahmias.** 1979. Herpes simplex virus glycoproteins: participation of individual herpes simplex virus glycoprotein antigens in immunocytolysis and their correlation with previously identified glycoproteins. J. Virol. **32**:741–748.
28. **Paul, P. S., W. L. Mengeling, and E. C. Pirtle.** 1982. Differentiation of pseudorabies (Aujeszky's disease) virus strains by restriction endonuclease analysis. Arch. Virol. **73**:193–198.
29. **Pizer, L. I., G. H. Cohen, and R. J. Eisenberg.** 1980. Effect of tunicamysin on herpes simplex virus glycoproteins and infectious virus production. J. Virol. **34**:142–153.
30. **Rigby, P. W. J., M. Dieckman, C. Rhoades, and P. Berg.** 1977. Labeling deoxyribonucleic acid to high specific activity in vitro by nick translation with DNA polymerase I. J. Mol. Biol. **113**:237–251.
31. **Roizman, B.** 1982. The family herpesviridae: general description, taxonomy, and classification, p. 1–23. *In* B. Roizman (ed.), The herpesviruses. Plenum Press, New York.
32. **Rubenstein, A. S., and A. S. Kaplan.** 1975. Electron microscopic studies of the DNA of defective and standard pseudorabies virions. Virology **66**:385–392.
33. **Ruyechan, W. T., L. S. Morse, D. M. Knipe, and B. Roizman.** 1979. Molecular genetics of herpes simplex virus. II. Mapping of the major viral glycoproteins and of the genetic loci specifying the social behavior of infected cells. J. Virol. **29**:677–697.
34. **Scherba, G., D. P. Gustafson, C. L. Kanitz, and I. L. Sun.** 1978. Delayed hypersensitivity reaction to pseudorabies virus as a field diagnostic test in swine. J. Am. Vet. Med. Assoc. **173**:1490–1493.
35. **Shen, Y.-M., R. R. Hischhorn, W. E. Mercer, E. Surmacz, Y. Tsutsui, K. J. Soprano, and R. Baserga.** 1982. Gene transfer: DNA microinjection compared with DNA transfection with a very high efficiency. Mol. Cell. Biol. **2**:1145–1154.
36. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**:503–517.
37. **Spear, P. G.** 1976. Membrane proteins specified by herpes simplex viruses. I. Identification of four glycoprotein precursors and their products in type 1-infected cells. J. Virol. **17**:991–1008.
38. **Stinski, M. F.** 1978. Sequence of protein synthesis in cells infected by human cytomegalovirus: early and late virus-induced polypeptides. J. Virol. **26**:686–701.
39. **Stow, N. D., J. H. Subak-Sharpe, and N. M. Wilkie.** 1978. Physical mapping of herpes simplex virus type 1 mutations by marker rescue. J. Virol. **28**:182–192.
40. **Straus, S. E., H. S. Aulakh, W. T. Ruyechan, J. Hay, T. A. Casey, G. F. Vande Woude, J. Owens, and H. A. Smith.** 1981. Structure of varicella-zoster virus DNA. J. Virol. **40**:516–525.
41. **Swaney, L. M.** 1976. Susceptibility of a new fetal pig kidney cell line (MVPK-1) to foot-and-mouth disease virus. Am. J. Vet. Res. **37**:1319–1322.
42. **Vestergaard, B. F.** 1980. Herpes simplex virus antigens and antibodies: a survey of studies based on quantitative immunoelectrophoresis. Rev. Infect. Dis. **2**:899–913.
43. **Walboomers, J. M. M., and J. T. Schegget.** 1976. A new method for the isolation of herpes simplex virus type 2 DNA. Virology **74**:256–258.
44. **Watson, R. J., J. H. Weis, J. S. Salstrom, and L. W. Enquist.** 1982. Herpes simplex virus type 1 glycoprotein D gene: nucleotide sequence and expression in *Escherichia coli*. Science **218**:381–384.
45. **Weis, J. H., L. W. Enquist, J. S. Salstrom, and R. J. Watson.** 1983. An immunologically active chimaeric protein containing herpes simplex virus type 1 glycoprotein D. Nature (London) **302**:72–74.