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P. E. Pellett
The University of Chicago

K. G. Kousoulas
The University of Chicago

L. Pereira
The University of Chicago

B. Roizman
The University of Chicago

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Anatomy of the Herpes Simplex Virus 1 Strain F Glycoprotein B Gene: Primary Sequence and Predicted Protein Structure of the Wild Type and of Monoclonal Antibody-Resistant Mutants

PHILIP E. PELLETT,[1] KONSTANTIN G. KOUSOULAS,[2] LENORE PEREIRA,[2] AND BERNARD ROIZMAN[1]*

Marjorie B. Kovler Viral Oncology Laboratories, University of Chicago, Chicago, Illinois 60637,[1] and Viral and Rickettsial Disease Laboratories, Department of Health Services, California Department of Health, Berkeley, California 94704[2]

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In this paper we report the nucleotide sequence and predicted amino acid sequence of glycoprotein B of herpes simplex virus 1 strain F and the amino acid substitutions in the domains of the glycoprotein B gene of three mutants selected for resistance to monoclonal antibody H126-5 or H233 but not to both. Analyses of the amino acid sequence with respect to hydropathicity and secondary structure yielded a two-dimensional model of the protein. The model predicts an N-terminal, 29-amino-acid cleavable signal sequence, a 696-amino-acid hydrophilic surface domain containing six potential sites for *N*-linked glycosylation, a 69-amino-acid hydrophobic domain containing three segments traversing the membrane, and a charged 109-amino-acid domain projecting into the cytoplasm and previously shown to marker rescue glycoprotein B *syn* mutations. The nucleotide sequence of the mutant glycoprotein B DNA fragments previously shown to marker transfer or rescue the mutations revealed that the amino acid substitutions cluster in the hydrophilic surface domain between amino acids 273 and 305. Analyses of the secondary structure of these regions, coupled with the experimentally derived observation that the H126-5- and H233-antibody cognitive sites do not overlap, indicate the approximate locations of the epitopes of these neutralizing, surface-reacting, and immune-precipitating monoclonal antibodies. The predicted perturbations in the secondary structure introduced by the amino acid substitutions correlate with the extent of loss of reactivity with monoclonal antibodies in various immunoassays.

The long-term objective of this study is to dissect the domains of a herpes simplex virus 1 (HSV-1) gene. We selected the glycoprotein B (gB) gene for several reasons. First, gB appears to be essential for production of infectious virus inasmuch as *ts* mutants mapping in that gene have been isolated (11, 25, 34, 36). Second, the phenotypes of the mutants obtained to date suggest that the gB gene has multiple functions. Thus, the gene may be essential for penetration of the virus into the cells (34, 54). The observations that *syn* loci map in the domains of gB (13, 30, 35, 51) and of other genes (35, 45a, 48) suggest that gB interacts with several different proteins and that mutations in any of these proteins may cause aberrant interaction, manifest by the *syn* phenotype (51). In addition, the wild-type virus gB exists as a dimer (54), and hence it contains cognitive sites for itself as well as for other proteins. The preeminent reason for the selection of the gB gene is that its product is incorporated into membranes and appears on the surface of both virions and infected cells. Because of this property, antibody to gB can be used to select mutants in that gene.

In a preceding paper we described a panel of distinct monoclonal antibodies to gB, a series of spontaneous and mutagen-induced mutants nonreactive in at least one immune reaction with two monoclonal antibodies to gB, preliminary mapping of the mutations by marker rescue or marker transfer, and a technique for rapid selection of mutants reactive or nonreactive with specific monoclonal antibodies (30). Those studies laid the groundwork for the analyses in this paper. We report here the nucleotide sequence of the domain of the gB gene of HSV-1 strain F [HSV-1(F)], the specific amino acid substitutions in three of the mutants selected for loss of reactivity with two monoclonal antibodies and previously described, a model of the structure of gB, and the predicted changes in the structure of gB induced by the mutations. We should note that after the sequencing of the wild-type HSV-1(F) gB gene was completed, a paper describing the nucleotide sequence of HSV-1 strain KOS [HSV-1 (KOS)] gB was published by Bzik et al. (10). Although the differences between the sequences of KOS gB and F gB are minor, there are significant differences in the interpretation of the structural consequences of the deduced amino acid sequence.

MATERIALS AND METHODS

Reagents. Restriction enzymes were obtained from Bethesda Research Laboratories, Gaithersburg, Md.; Pharmacia P-L Biochemicals, Piscataway, N.J.; and New England Biolabs, Beverly, Mass.; and were used according to the instructions of the suppliers. T4 DNA ligase, *Escherichia coli* DNA polymerase I large fragment (Klenow fragment), and calf intestinal alkaline phosphatase were from Boehringer Mannheim Biochemicals, Indianapolis, Ind. T4 DNA polymerase was from Bethesda Research Laboratories, sequencing primers were from New England Biolabs, and deoxy- and dideoxy-nucleotide triphosphates were from Pharmacia P-L Biochemicals.

Strains and vectors. Plasmid vectors pUC8 and pUC9 and their host JM83 (58) were obtained from D. J. Messing through Bethesda Research Laboratories.

Viruses, cells, and media. The properties of HSV-1(F), the parent wild-type virus used in these studies, were described elsewhere (15). The isolation and properties of the monoclonal antibody nonreactive mutants and the procedures for

* Corresponding author.

their propagation and for preparation of their DNA were as described previously (30).

Recombinant DNA methods. The procedures for generating the panel of clones used in sequencing the gB gene, as well as for cloning the mutant viral DNAs, were as previously described (30). The *Ava*I sites (see Fig. 1) were used to clone the appropriate regions of R233/S9 and R126/B1 mutant DNAs as blunt-end fragments appropriate for sequencing into the *Sma*I site of pUC9. Because the *Ava*I site at nucleotide 1792 in mutant R126/S8 was lost, the appropriate DNA fragments of that mutant were cloned from *Nar*I and *Nar*I-*Pst*I digests (see Fig. 1) into pUC9.

DNA sequencing. All sequencing was performed by the dideoxy technique (3, 53), with plasmid DNA as a template (61) and pUC8 and pUC9 as vectors. DNA was purified on glass powder (59) after an acid-base lysis of the bacteria (4). Briefly, the alcohol precipitate from the lysis of a 100-ml culture of bacteria was resuspended in 3 ml of TE (10 mM Tris–1 mM EDTA, pH 8), to which 9 ml of 8 M $NaClO_4$ was added, followed by 300 μl of glass powder slurry prepared by the method of Patterson (personal communication). After 1 to 2 h on ice, the glass was pelleted and washed once with the salt solution and then twice with 50% EtOH–0.1 M NaCl–1 mM EDTA. To minimize nicking and shearing of the DNA, vortexing was kept to a minimum. The DNA was eluted from the glass by incubation with 1 ml of TE at 37°C for 30 min.

Approximately 0.6 pmol of subsequently linearized plasmid was mixed with an appropriate dilution of primer (normal M13 sequencing primer or M13 reverse sequencing primer) in a total volume of 12 μl at 1× HIN (50 mM NaCl, 10 mM $MgCl_2$, 10 mM Tris [pH 7.4], 10 mM dithiothreitol). The mixture was boiled for 5 min, quick-chilled on dry ice-ethanol, and then allowed to anneal in a 24°C water bath for 20 to 40 min before sequencing in a standard reaction. For approximately the first half of the project, [α-^{32}P]dATP (New England Nuclear Corp., Boston, Mass.) was used, and for all subsequent studies, [α-^{35}S]dATP from Amersham Corp., Arlington Heights, Ill., was used (3). A 3:1 mixture of 2 mM dITP–1 mM dGTP was substituted for 1 mM dGTP when making up the reaction mixtures, to resolve gel compressions (39, 52).

Gels were bonded to the glass plates, fixed, and dried by the method of Garoff and Ansorge (24), and in the final stages of the study, gradient buffer sequencing gels were used (3). Gels were read, and the data were entered twice into a General Automations SPC-16/45 computer, with a sonic digitizer linked to software which allowed immediate comparison of the two entries to ensure the accuracy of the transcription.

Computer analysis of the sequences. The program of Queen and Korn (46), running on an IBM 3081D; the sequence analysis package obtained from Hugo Martinez, University of California at San Francisco Biomathematics Computation Laboratory, running on a Hewlett-Packard 9000; and hydropathic analysis and graphing programs written by P.E.P., also running on a Hewlett-Packard 9000, were used in the analysis of the DNA sequence and in the modeling of the structure of gB. Amino acids were designated by a conventional three-letter or single-letter code; the number of the amino acid is indicated throughout the text.

RESULTS

DNA and amino acid sequence analyses. (i) Nucleotide sequence of the HSV-1(F) gB gene. To sequence the gB gene, two series of DNA fragments were generated by the DNase I deletion subcloning strategy of Frischauf et al. (21). Each series was generated from a 6-kilobase-pair *Bam*HI-*Sal*I cloned DNA fragment containing the entire domain of the gB gene. In each series, the deletions extended from one terminus toward the other in increments of ca. 300 base pairs. The generation and properties of the cloned DNA fragment bank were described elsewhere (30) in connection with the mapping of monoclonal antibody-resistant mutations in the HSV-1(F) gB gene. The terminal 250 to 300 bases of the cloned DNA fragments were sequenced as described above; both strands were sequenced in their entirety (Fig. 1). The diagram of the sequenced region (Fig. 1) shows the position and orientation of the sequence relative to the HSV prototype orientation, a restriction map, the sequencing strategy, and potential translation initiation and termination codons in each of the six reading frames. Figure 2 shows the nucleotide and amino acid sequence of the domain of the gB gene.

The domain of the gB gene has previously been defined by the mapping of *ts* mutants, the syncytial phenotype (syn), mutations affecting reactivity with monoclonal antibody, and mRNA (10, 13, 27, 28, 30, 51). The mRNA encoding gB has been shown to be transcribed from right to left in the prototype orientation (10, 27, 51), whereas for consistency with sequence and structural depictions later in the text it is shown left to right in Fig. 1. After this work was completed, the sequence of HSV-1(KOS) gB also was published (10). A detailed description of the sequence differences is presented in the legend to Fig. 2, and a discussion of the differences in the interpretation of the structural consequences of the deduced amino acid sequence is presented in the discussion. The results (Fig. 1) indicate that the largest open reading frame in phase 3 extending from left to right from the translation initiation site at nucleotide 795 to the termination site at nucleotide 3504 is the only reading frame compatible with the known size of the protein and genetic markers mapped within the domain of the gB gene. The only other large open reading frame of sufficient size (phase 2 reading in the opposite direction) is excluded by being anticomplementary to gB mRNA (27); it would yield a protein significantly smaller than gB and would lack the domain of the *syn* mutations. The open frame in phase 3 reading from left to right and encoding the nucleotide sequence of gB overlaps at its 5′-terminal 332 nucleotides of an open frame in phase 2 reading in the same direction. The nucleotide sequence suggests that the region may contain the C-terminal amino acids of the protein associated with a recently identified complementation group and a 5.6-kilobase mRNA mapping between the ICP 8 and gB genes (14, 27, 43). We have extended the open frame leftward to 261 amino acids. It is noteworthy that the translation of this putative protein terminates just 10 nucleotides before the translation initiation site of gB and that its coding sequence spans the upstream promoter and regulatory region of gB.

Several sequences associated with transcription and translation were found within and adjacent to the reading frame of gB. They specifically are as follows.

(a) A TATA box with the sequence ATATATT was present at nucleotides 481 to 487, and the sequence CACA previously associated with the site of initiation of transcription (8) was found, as predicted, beginning with nucleotide 508.

(b) The sequence ATTG at nucleotide 399 reads CAAT on the opposite strand; inasmuch as it is 100 base pairs upstream from the transcription initiation site, it could serve as a CAT box (8).

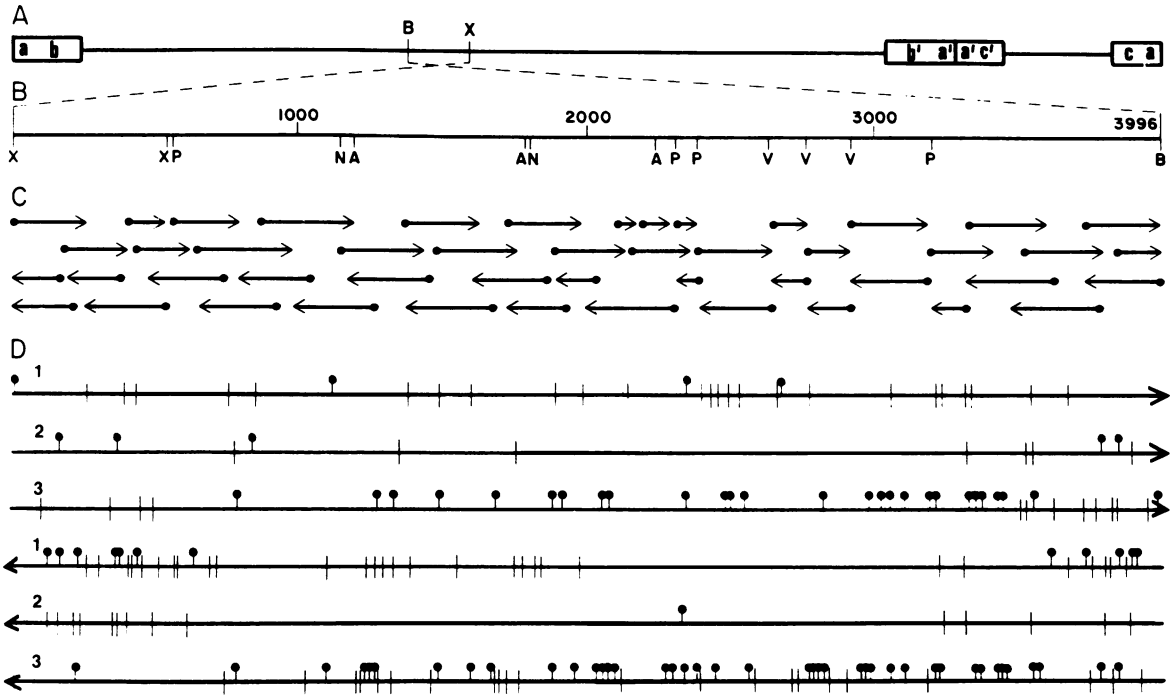

FIG. 1. Domain of the HSV-1(F) gB gene. A, DNA sequence arrangement of HSV-1(F) DNA; B, partial restriction enzyme map of the sequenced region. For convenience, the sequenced region is shown inverted relative to its orientation in the P arrangement of HSV-1 DNA. The enzymes indicated are: *Ava*I, A; *Bam*HI, B; *Nar*I, N; *Pst*I, P; *Pvu*II, V; and *Xho*I, X. The *Ava*I and *Nar*I sites shown were those that were used for subcloning cloned DNA fragments from DNAs of monoclonal antibody-resistant mutants. The numbers indicate the distance in nucleotides from the first nucleotide of the left *Xho*I recognition site. C, Map of the sequencing overlaps. The dot at the beginning of each line indicates the position of the first nucleotide of a sequenced clone. The arrow indicates the direction and the approximate extent of sequence read from that clone. D, Map of potential translation initiation codons (ball on stem) and termination codons (stems) in each of the three reading frames in both directions, as indicated by the arrows.

(c) The sequence GGGGGCGGGGGGCTCGGGTGC-TGATTGG near this CAT box resembles the second distal signal of the thymidine kinase gene (37) but differs from that of the thymidine kinase gene in that the hexanucleotide core sequence (38) is in an orientation inverted relative to that of the thymidine kinase gene.

(d) Two polyadenylation signals, AATAAA (2), were present downstream from the gB coding region. The first, beginning with nucleotide 3523, was 16 nucleotides downstream from the translation termination codon at nucleotide 3504, whereas the second began at nucleotide 3766. One or both of these sites could be used for termination of gB mRNA and of the 5.6- and 10-kilobase mRNAs mapped to this region (10, 27, 47). A sequence at position 3818 could serve as the polyadenylic acid site for the family of λ and β/λ messages which initiate outside of *Bam*HI-G and are transcribed from the strand opposite the one used by gB (27). These mRNAs were previously shown to terminate in the region defined by the *Sac*I site at position 3237 and the terminal *Bam*HI site 764 nucleotides away (27).

(e) Efficient initiation of translation is thought to require a complementary interaction between the mRNA sequence and a portion of 18S rRNA (26, 56). A complementary sequence with 8 of 10 bases matching the rRNA sequence was found at nucleotide 732, i.e., 63 bases upstream from the first AUG. This AUG is likely to be the initiation codon for two reasons. First, the modified scanning hypothesis of translation initiation (31) predicts that the first AUG in a mRNA will serve as the initiation codon provided the local environment is suitable. According to Kozak (31, 32), the gB DNA sequence GCCATGCGC qualifies this AUG as an efficient initiation site. Second, the sequence of amino acids encoded by the DNA stretch immediately after this codon was the only one within the gB gene that conforms with the predicted structure of a signal sequence as discussed below. Bzik et al. (10) also identified this AUG as the translation initiation codon.

(f) The gB stop codon TGA was found at nucleotide 3504 and was followed by additional stop codons in all three reading frames.

The molecular weight of the 903-amino-acid translation product of the gB gene is 100,113; after cleavage of the predicted 29-amino-acid signal sequence, the predicted molecular weight of the 874-amino-acid nonglycosylated protein would be 97,028. These molecular weights are well within the range of the published apparent molecular weight of gB (6, 41, 43, 57, 63).

(ii) Nucleotide sequences of DNA fragments from the domain of the gB gene carrying mutations conferring resistance to gB-specific monoclonal antibodies. In previous studies we described several HSV-1(F) mutants resistant to monoclonal antibodies in at least one immune reaction (30). Of these mutants, three were chosen for sequencing of the mutated site. The spontaneous mutant R126/S8 was resistant to neutralization by monoclonal antibody H126-5; however, the mutant gB reacted with this antibody in biotin-avidin-enhanced surface immunoassays and in immune precipitation tests. The spontaneous mutant R233/S9 was resistant to neutralization by monoclonal antibody H233; the mutant gB reacted with this monoclonal antibody in biotin-avidin-enhanced surface immunoassays and in immune precipitation tests. Both spontaneous mutants reacted with the heterolo-

FIG. 2. Nucleotide sequence and predicted amino acid sequence of HSV-(F) gB. In the P arrangement of HSV-1(F) DNA, gB is transcribed from right to left. Nucleotide 1 is the first nucleotide of the left *Xho*I site as shown in Fig. 1, and nucleotide 3996 is the last nucleotide of the *Bam*HI site at the left end of the *Bam*HI fragment G of HSV-1(F) in the P arrangement. The TATA box and presumptive transcription initiation sites are underlined with solid lines, and potential sites for N-linked glycosylation are underlined by a thick line with diagonal stripes. In relation to the sequence shown, the published nucleotide sequence of the HSV-1(KOS) gB gene (10) begins at nucleotide 248 and terminates at nucleotide 3996. Both HSV-1(F) and HSV-1(KOS) have an identical number of amino acids. The amino acid sequences differ at the following positions [shown as the KOS amino acid at the corresponding HSV-1(F) amino acid position]: His_{-28}, Pro_{-24}, Ser_{-23}, Trp (between −23 and −22), Arg_{-21}, Pro_{+37}, Thr_{+49}, Thr_{+283}, Val_{+523}, and Leu_{+726}. In addition, the deletion of two bases in KOS relative to HSV-1(F) at nucleotide 915 resulted in a reading frameshift for a total of 19 amino acids (amino acids 12 to 30) until a one-base deletion in the KOS sequence relative to that of F at position 962 returned the amino acid sequences to a homologous alignment. In addition to the three nucleotides deleted in the coding sequence indicated above (nucleotide 915, 916, and 962), KOS lacks a nucleotide (3958) downstream from the coding region. KOS contains 12 nucleotides absent in HSV-1(F); 3 are located between HSV-1(F) nucleotides 811 and 812 and code for Trp, and the remainder are in the 3′ noncoding region. In addition, there are 37 nucleotide substitutions, of which 27 are in the coding region.

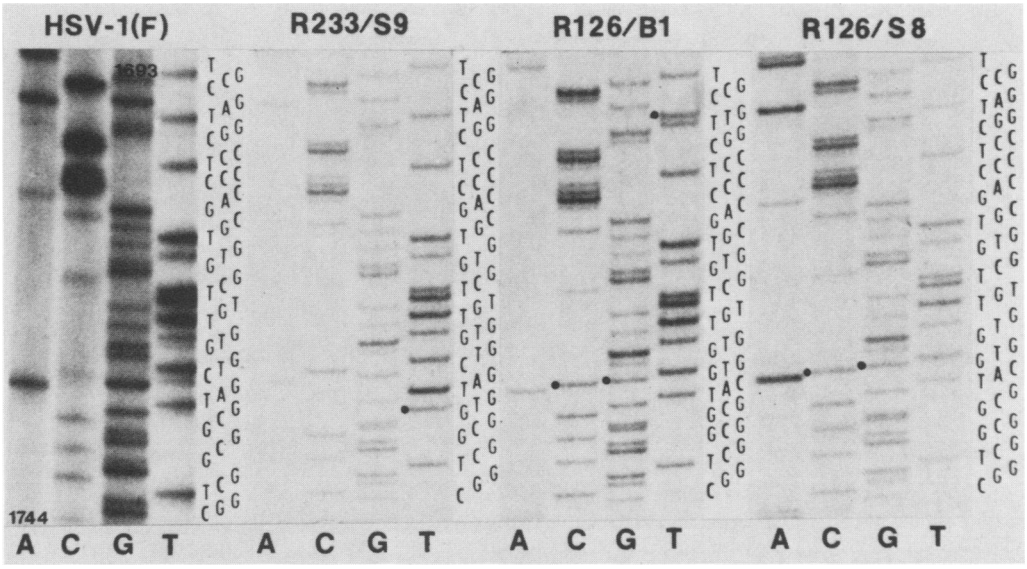

FIG. 3. Autoradiograms of portions of DNA sequencing gels showing some of the HSV-1(F) nucleotide sequence and the corresponding sequences of three mutants selected for resistance to monoclonal antibodies in at least one immunoassay. The nucleotide sequence is shown for nucleotides 1744 to 1693, reading on the strand complementary to the one shown in Fig. 2. The bases altered in the mutants relative to the wild-type virus are indicated on the autoradiograms with dots. Letters A, C, G, and T under each lane indicate which chain-terminating dideoxy-nucleotide triphosphate was present in the sequencing reaction loaded in that lane. The HSV-1(F) sequence was obtained with [α-^{32}P]dATP, and autoradiography was done on a wet nongradient gel. The mutant DNA sequences were obtained with [α-^{35}S]dATP, and the buffer gradient gel was fixed and dried before autoradiography (3).

gous monoclonal antibodies in all three tests. Moreover, the resistance phenotype of both mutants was transferable to wild-type HSV-1(F) by the corresponding 1,745-base-pair *Pst*I fragments extending from nucleotides 562 to 2307. Bromodeoxyuridine-induced mutant R126/B1 failed to react with monoclonal antibody H126-5 in all three immunoassays, although it reacted in all three tests with the heterologous H233 monoclonal antibody. The phenotype of this mutant was mapped in marker rescue tests with wild-type DNA fragments to nucleotides 1482 to 1869.

The results of the nucleotide sequencing of the relevant regions are shown in Fig. 3 and 4. R126/S8 contains substitutions at amino acids 283, 305, and 443; R126/B1 contains substitutions at amino acids 273 and 283; and R233/S9 contains a single substitution at amino acid 285.

Modeling of the gB structure. The secondary structure of gB was predicted by a combination of the hydropathic analysis of Kyte and Doolittle (33), the empirically based secondary-structure analyses of Chou and Fasman (11) and Garnier et al. (23), and the helical wheel models of Schiffer

Virus	Nucleotide and Predicted Amino Acids Sequence
HSV-1(F)	272 286 303 307 441 449 Gly Tyr Arg Glu Gly Ser His Thr Glu His Thr Ser Tyr Ala Ala Lys Ala Arg Ala Thr Glu Gln Ser Arg Lys GGC TAC CGG GAG GGG TCG CAC ACC GAA CAC ACC AGC TAC GCC GCC AAG GCC CGG GCC ACG GAG CAG AGC CGC AAG
R126/S8	283 305 443 Gly Tyr Arg Glu Gly Ser His Thr Glu His Thr THR Tyr Ala Ala Lys Ala GLN Ala Thr Glu Gln ASN Arg Lys GGC TAC CGG GAG GGG TCG CAC ACC GAA CAC ACC Acg TAC GCC GCC AAG GCC CaG GCC ACG GAG CAG AaC CGC AAG
R126/B1	273 283 Gly ASN Arg Glu Gly Ser His Thr Glu His Thr THR Tyr Ala Ala Lys Ala Arg Ala Thr GGC aAC CGG GAG GGG TCG CAC ACC GAA CAC ACC Acg TAC GCC GCC AAG GCC CGG GCC ACG
R233/S9	285 Gly Tyr Arg Glu Gly Ser His Thr Glu His Thr Ser Tyr THR Ala Lys Ala Arg Ala Thr Glu Gln Ser Arg Lys GGC TAC CGG GAG GGG TCG CAC ACC GAA CAC ACC AGC TAC aCC GCC AAG GCC CGG GCC ACG GAG CAG AGC CGC AAG

FIG. 4. Nucleotide sequence and predicted amino acid sequence of DNA regions of HSV-1(F) and mutant viruses capable of inducing an alteration in the reactivity of gB in marker transfer or marker rescue tests. Numbers are amino acid numbers. Amino acid number 1 is the first amino acid after the putative site of the signal sequence cleavage. Substituted nucleotides are shown in lowercase letters, whereas substituted amino acids are shown in uppercase letters.

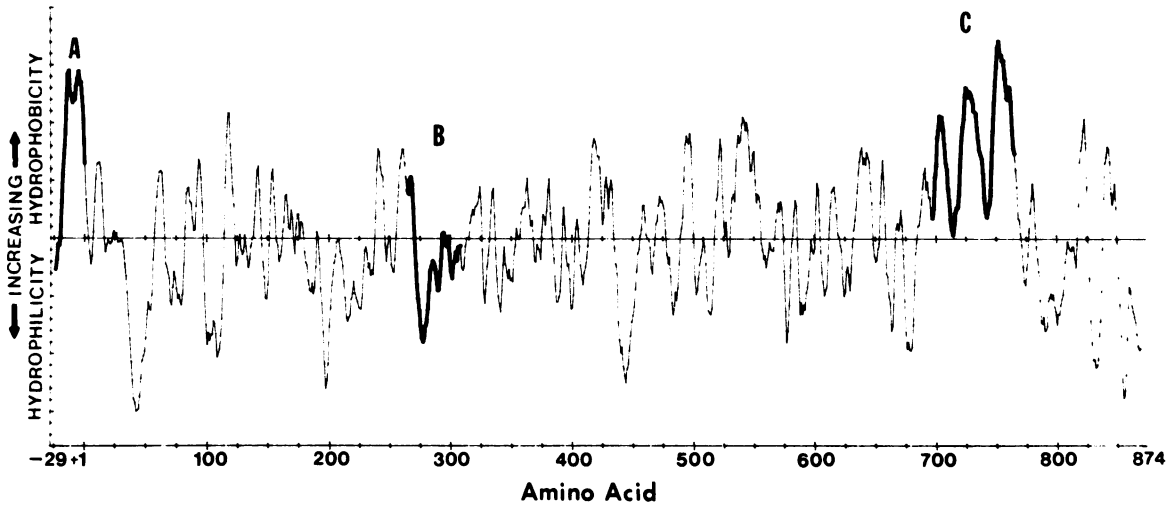

FIG. 5. Hydropathic profile of gB. The determination of relative hydropathy as a function of position along the amino acid sequence was done as described by Kyte and Doolittle (33), with a moving window of seven amino acids. The resulting profile was then smoothed by taking its average in a moving window of three points, and the smoothed profile was plotted. The x-axis is drawn so that points above it are of greater than average hydrophobicity as defined by Kyte and Doolittle (33). The thickened lines identify the predicted cleaved signal sequence (A), the domain of the molecule whose secondary structure is altered in the monoclonal antibody-resistant mutants as described later in the text (B), and the three hydrophobic peaks of the predicted membrane-traversing domain (C). Amino acid 1 is the amino acid predicted to follow the signal peptidase cleavage site.

and Edmundson (55). The hydropathic profile and a model of the secondary structure of gB are shown in Fig. 5 and 6, respectively. The amino acid sequence predicts a signal sequence (shown in Fig. 5 but not in Fig. 6), a hydrophobic membrane-spanning domain, a cytoplasmic domain, and a hydrophilic domain predicted to project from the surfaces of virion envelopes and membranes of infected cells. It is convenient to describe each domain separately.

(i) The signal sequence domain. Since gB is a membrane-bound glycoprotein, it could be predicted to possess a membrane insertion (signal) sequence and a potentially independent membrane anchor region. A region extending from amino acids −29 to −1 has the characteristics of a cleavable signal sequence and therefore should be unable to serve as an anchor sequence. The findings specifically were as follows.

(a) A hydrophobic sequence of 17 amino acids containing a single polar residue (Thr) was found from Trp_{-19} to Ala_{-3}. This region is of sufficient length to serve as the hydrophobic core of a signal sequence but is not long enough to serve as an anchor (17, 45, 60).

(b) A characteristic structure of signal sequences is the presence of a hydrophobic region predicted by Chou and Fasman analysis to have both α-helical- and β-sheet-forming potential (45). Hydrophobic sequences with such potentials are found in β-sheet conformation when analyzed by circular dichroism in aqueous solvents and in α-helical conformation when analzyed in nonpolar solvents (50), even if, as is the case with gB, the β-sheet potential is higher than the α-helical potential.

(c) The sequence Val_{-13}-Val_{-12} was found at positions 7 and 8 of the hydrophobic core. Both this sequence and its position within the core are highly conserved in signal sequences of both eucaryotes and procaryotes (45).

(d) Another characteristic of signal sequences is that the hydrophobic sequence is preceded by positively charged residues (45); in gB, the hydrophobic core was preceded by a 10-amino-acid region containing three positively charged amino acids.

(e) The sequence Ala_{-3}-Ser_{-2}-Ala_{-1} corresponded exactly with the sequence Ala-X-Ala, which is the most common sequence found preceding the signal peptidase cleavage site (45, 60). Ser is commonly found in the X position.

(f) A predicted β-turn is usually found before or after the cleavage site (45); the Ala_{-3}-Ser_{-2}-Ala_{-1} sequence of gB was followed immediately by a β-turn.

On the basis of these considerations, Ala_{+1} is predicted to be the N-terminal amino acid of the mature protein.

(ii) The hydrophobic transmembrane domain. This domain is readily apparent in the hydropathic profile (Fig. 5) as three hydrophobic peaks, each ca. 20 amino acids in length. The hypothesis that this domain contains three antiparallel segments traversing the membrane, each segment being connected to the next by a short turn region, is supported by the following considerations.

(a) Arguments for the necessity of membrane-traversing segments to be in a helical conformation have been presented previously (17). The Chou and Fasman analyses of the three segments predict that the first (Ala_{+697} to Arg_{+717}) and the third (Phe_{+745} to Phe_{+765}) segments have an α-helical structure. The second segment (Val_{+722} to Ser_{+742}) has both α-helical and β-sheet potential, but its β-potential is higher than its α-helical potential. As was indicated above with respect to the likely conformation of the signal sequence, such regions are likely to adopt an α-helical conformation in the nonpolar environment of the lipid bilayer. A striking consequence of the model in Fig. 6 is that in a helical wheel arrangements (not shown) (55), small side-chain amino acids would cluster on one face of segments 1 and 3, whereas both small side-chain amino acids and polar residues (Ser) would cluster on one face of segment 2. Such an arrangement would permit these faces to pack closely and form intersegment hydrogen bonds. In such an arrangement the faces exposing larger nonpolar residues of the triple helical cluster would be oriented toward the lipid environment of the membranes.

(b) The turn between segments 1 and 2 is formed by Ala_{+718}-Val_{+719}-Gly_{+720}-Lys_{+721} immediately after

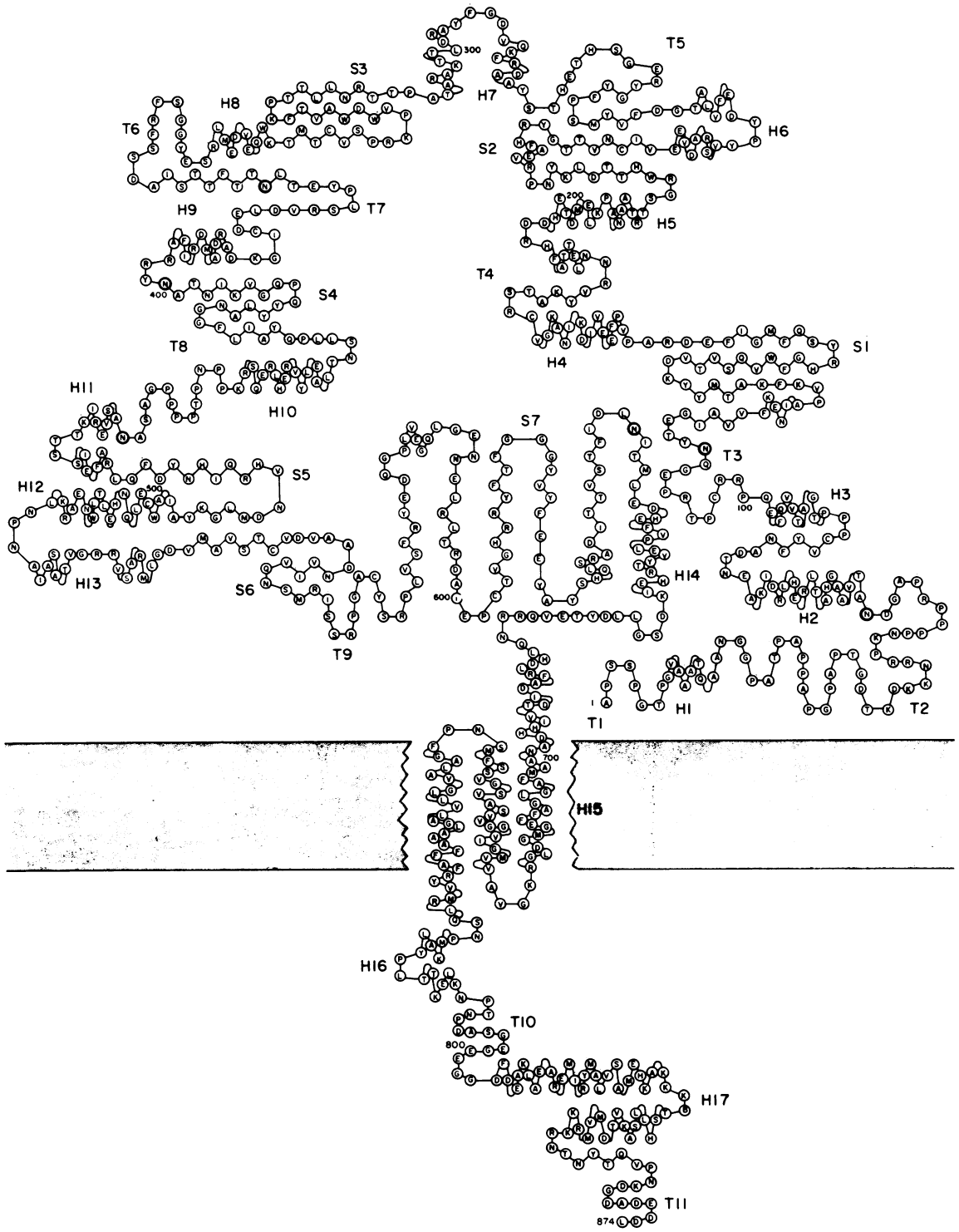

FIG. 6. Predicted membrane orientation and secondary structure of gB. The prediction was based on a combination of hydropathic analysis (33), empirically based secondary structure analysis (11, 23), and helical wheel analysis (55). Two domains containing internal Pro residues are shown as helical domains, notwithstanding the Chou and Fasman predictions, because the probability of these domains forming a helix was greater than five times their probability of being found in any other structure according to the predictions of Garnier et al. (23). α-Helical regions are indicated as helices, β-sheets are indicated as zig-zags, and chain direction changes were drawn only at predicted β-turns. For the purpose of discussion and reference, the major helical, sheet, and turn domains were numbered from the amino terminus and prefixed with the letters H, S, and T, respectively. Sites for potential *N*-linked glycosylation are indicated with thickened circles. Amino acids are numbered in 100's, beginning at the amino acid predicted to follow the signal peptidase cleavage site. The membrane is indicated by the stippled band. A standard one-letter amino acid code was used throughout: Ala, A; Arg, R; Asn, N; Asp, D; Cys, C; Gln, Q; Glu, E; Gly, G; His, H; Ile, I; Leu, L; Lys, K; Met, M; Phe, F; Pro, P; Ser, S; Thr, T; Trp, W; Tyr, Y; Val, V.

Arg_{+717}. At least part of the turn projects outside the membrane. The turn between segments 2 and 3 is formed by Ser_{+742}-Asn_{+743}-Pro_{+744}-Phe_{+745}; Ser_{+742} and Phe_{+745} are within the helical structure and are likely to be within the membrane.

(c) Segment 1 contains three charged residues (Glu_{+710}, Asp_{+714}, and Arg_{+717}). Asp_{+714} and Arg_{+717} would occur on the same side of the helix and would be able to form a charge-neutralizing salt bridge. The thermodynamic analyses of Engelman and Steitz (17) predict that the thermodynamic stress is not so prohibitive as to preclude their presence is the membrane.

(iii) The cytoplasmic domain. Because the hydrophobic region is predicted to traverse the membrane an odd number of times, the immediately adjacent domains of the gB must be located on opposite sides of the membrane. The placement of the C-terminal domain on the underside of the membrane is based on the absence of effective glycosylation sites in the C-terminal region and the presence of both effective glycosylation sites (Asn-X-Ser/Thr, where X is not Pro) (49) and epitopes for neutralizing monoclonal antibodies on the N-terminal domain. The turn domains T10 and T11 and the helical domains H16 and H17 (Fig. 6) are shown as predicted from their amino acid sequence. Of the 109 amino acids in this domain, 39 are charged, and the net charge is positive.

(iv) The hydrophilic surface domain. The hydrophilic domain N terminal to the hydrophobic trans-membrane region is shown as predicted from the amino acid sequence in an unfolded, two-dimensional interpretation with little regard to possible three-dimensional interactions. The amino acid sequence predicts at least 7 β-sheet domains, 11 major turn domains, and 17 helical domains with more than 10 amino acids. It is of interest that the six efficient glycosylation sites are located at the junction of helical or β-sheet structures with predicted turns, and hence they may be exposed to the external environment of the molecule.

(v) The predicted secondary structures of wild-type and mutated H233 and H126-5 monoclonal antibody cognitive sites. Relevant to the consideration of the structure of H233 and H126-5 monoclonal antibody cognitive sites are the following considerations.

(a) The immune assays used in the characterization of the monoclonal antibody-resistant mutants previously published (30) were neutralization, biotin-avidin-enhanced surface immunoassay, and immune precipitation tests. Analyses of the mutants revealed a hierarchical order of sensitivity of the tests to perturbations in structure introduced by mutations. Thus, mutants which failed to react with a monoclonal antibody in immune precipitation tests also failed to react in biotin-avidin-enhanced surface immunoassays and in neutralization tests. Conversely, mutants selected for resistance to neutralization reacted with the corresponding antibody in all other tests. These results indicate that mutations which yielded nonimmune precipitating gB introduced greater perturbations than those that rendered the gB nonreactive in biotin-avidin-enhanced surface immunoassays. Minor perturbations which affected neither of these reactions could abolish neutralization.

(b) Mutant R126/B1 reacted with H233 but not with H126-5 monoclonal antibodies in immune reactions, i.e., neutralization, biotin-avidin-enhanced surface immunoassays, and immune precipitation. Nucleotide sequence analysis (Fig. 4) showed that it contains two amino acid substitutions, i.e., Tyr_{+273} to Asn, and Ser_{+283} to Thr. The mutant was rescued by DNA fragments spanning both mutated

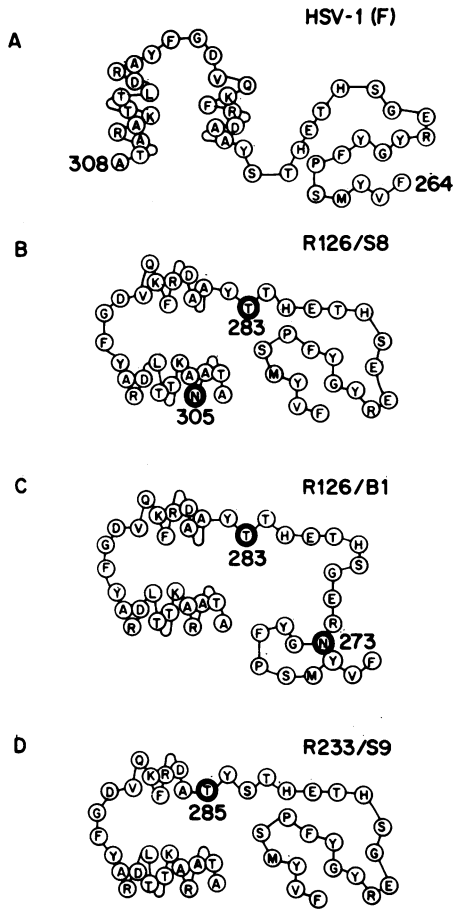

FIG. 7. Predicted secondary structure of the gB domains of H126-5 and H233 monoclonal antibody-resistant mutants mapped by marker transfer or rescue and containing amino acid substitutions. A, Predicted secondary structure of the HSV-1(F) gB for amino acids 264 to 308, as shown in Fig. 5; B, C, and D, predicted secondary structure of R126/S8, R126/B1, and R233/S9, respectively. The secondary structure alterations were determined by the method of Chou and Fasman (11) and are represented as described in the legend to Fig. 5. The substituted amino acids are indicated with thickened circles.

sites, but not by a DNA fragment containing the Thr_{+283} substitution three nucleotides from one of its termini (30). Mutant R126/S8 contains three substitutions, i.e., Thr for Ser_{+283}, and Gln for Arg_{+305}, and Asn for Ser_{+443}. This mutant reacted with H233 in all immune tests and with H126-5 in immune precipitations and biotin-avidin-enhanced immunoassays but not in neutralization tests. These results suggest that the cognitive (epitopic) site for H233 is different from the mutations in the R126/B1 and R126/S8 gB genes and that the major perturbation in the H126-5 cognitive site was introduced by the Asn_{+273} substitution rather than by the Gln_{+305} and Asn_{+443} substitutions, and hence the H126-5 cognitive site is located to the N-terminal side of Ser_{+283} rather than to the C-terminal side of that amino acid in the diagram of HSV-1(F) shown in Fig. 7. Secondary structure analyses of R126/S8 gB (Fig. 7B) predict that the substitution of Thr for Ser_{+283} would result in the loss of a β-turn and of one α-helical residue and the generation of a four-residue β-sheet. The substitutions of Gln for Arg_{+305} and Asn for Ser_{+443} do not grossly alter the predicted secondary structure of the H126-5 epitopic site in R126/S8, but the substitu-

tion of amino acids with different hydrogen-bonding capabilities or side chain size, or both, could still have an effect on epitopic conformation. The substitution of Thr for Ser_{+283} and the substitution of Asn for Tyr_{+273} in R126/B1 (Fig. 7C) could grossly perturb the intervening region because, in addition to the predicted loss of the β-turn and of one α-helix residue with the gain of a four-residue β-sheet seen in R126/S8, there would be the substitution of a β-turn for a four-residue β-sheet.

(c) Mutant R233/S9 contains a single substitution of Thr_{+385} for Ala. This mutant reacted in all three immunoassays with H126-5 and H233 monoclonal antibodies in biotin-avidin-enhanced surface immunoassays and immune precipitation tests. The observed perturbation is therefore minimal; because the mutation at amino acid 283 has no effect on the reactivity with H233, it could be predicted that the H233 cognitive site is different from amino acid 283 and is to the C-terminal side of Ala_{+285} in wild-type gB. The substitution of Thr for Ala_{+285} causes the loss of a predicted turn and two residues of an α-helix, with their replacement by a five-residue β-sheet (Fig. 7D).

It is of interest to note that of the three mutants sequenced, two (R126/B1 and R126/S8) contain multiple mutations. R126/S8 contains four altered nucleotides resulting in three amino acid substitutions (Fig. 4). This virus was selected for resistance to neutralization by monoclonal antibody H126-5 from a nonmutagenized plaque-purified stock of HSV-1(F) (30). R126/B1 contains three altered nucleotides, resulting in two amino acid substitutions. This virus was selected from a bromodeoxyuridine-mutagenized stock of plaque-purifiedHSV-1(F) for its failure to react in a biotin-avidin-enhanced surface immunoassay after an initial selection for resistance to neutralization by monoclonal antibody H126-5 (30). R233/S9, which contains a single nucleotide substitution resulting in one amino acid substitution, was selected by the same procedure as was mutant R126/S8, except the selecting monoclonal antibody was H233. There was no correlation of amino acid substitution frequency among the three mutants analyzed with respect to the method of selection used.

DISCUSSION

The model of gB based on the nucleotide sequence of the HSV-1(F) gB gene and presented in this report is tentative and useful only for its predictive features. At this time, however, the model is consistent with all of the features of gB amenable to an immediate test.

(i) The model predicts a hydrophilic surface domain containing glycosylation sites and epitopes of neutralizing antibodies, a hydrophobic transmembrane anchor region, and a hydrophilic positively charged cytoplasmic domain. We have no direct evidence that the hydrophobic region designated as the anchor sequence actually serves this purpose. However, its structure is consistent with those of transmembrane domains of other proteins (1, 62). Circumstantial evidence, however, supports this view. Thus, the *syn* mutation maps on the C-terminal side of this region, within the domain of the protein predicted to be in the cytoplasm. The other *syn* loci mapped to date are located in regions of the genome not known to specify surface proteins (5, 45a). The hypothesis that *syn* mutations result from aberrant complexes of several viral proteins (51) predicts therefore that some of the diverse proteins forming this putative complex must be located within the lipid bilayer or underneath the membrane. It is comforting therefore that the *syn* mutation in gB maps in the protein domain assigned to the cytoplasm. The nearest and only region with a structure capable of traversing the membrane is the one predicted by the model.

(ii) All of the predicted glycosylation sites are within domains of the gene accessible to the surface of the protein. However, it should be noted that evidence confirming the existence of six oligosaccharide chains in partially or fully processed gB is lacking.

(iii) The sites reactive with monoclonal antibodies H126-5 and H233 are in hydrophilic, exposed domains of the gB model. This is consistent with the observation that both H126-5 and H233 monoclonal antibodies efficiently neutralize the virus. It is of particular interest that resistance to one monoclonal antibody does not affect the reactivity of the epitope recognized by the other antibody, indicating that although the mutations map close to each other, the perturbation in sequence and conformation induced by mutations in one epitope are not of a sufficient magnitude to affect the reactivity of the other. The mapping of the epitopes of gB is in its infancy. In this paper we report the approximate domain of the cognitive site of monoclonal antibody H125-6 and the approximate location of the H233 monoclonal antibody epitope. Mapping of additional independently derived mutants resistant to H125-6 and H233 should define their epitopes more precisely. In addition, selection and characterization of mutants to type-specific and nonneutralizing monoclonal antibodies constituting the monoclonal antibody panel previously described (30) should help outline the surface of the tertiary structure of gB.

A striking feature of the model of gB structure is the predicted triple pass through the membrane. Somewhat similar arrangements of three membrane-spanning regions have been predicted for the E1 glycoprotein of a coronavirus (1) and for the hydrophobic subunits of the *E. coli* fumarate reductase (62). Although gB is a relatively large protein, its size is not so much greater than that of other membrane-bound glycoproteins which traverse the membrane only once for size alone to dictate the requirement for the presence of three interacting membrane-spanning segments. The functional significance of the triple membrane pass predicted by these studies remains obscure but is not likely to be trivial. Among the possible functions of membrane domains consisting of several closely packed helices is that of an ion channel. Such a function has been associated with several proteins whose structures have been shown or predicted to include multiple helical passes through the membrane (7, 9, 12, 16, 18–20, 28, 39, 40). A characteristic feature of such structures is the amphipathic character of the membrane-spanning segments, where one face of a membrane-spanning helix typically is nonpolar and the other face contains several polar and charged residues. Such amphipathic helices may then associate in the membrane through specific interactions between the faces containing polar and charged residues, with the nonpolar faces becoming oriented outward toward the membrane lipids, as we have predicted for gB. The resulting structure is a protected pore through which ionic traffic may be mediated with the ionic specificity and control of transport being a function of the specific structure.

The presence of machinery with the potential to modulate intracellular ionic concentrations in an infected cell is of interest because inversion of the transmembrane potential due to perturbation of intracellular ionic concentrations after infection by herpes viruses has been observed (22). Intracellular ionic perturbation as the basis for changes in the

translational machinery of the infected cell has been postulated but never proven. This possible function of gB is, fortuitously, testable.

A comparison of the published sequence of HSV-1(KOS) gB by Bzik et al. (10) with the sequence reported here for HSV-1(F) showed that in the region where both viruses were sequenced there are a total of 53 nucleotide differences which resulted in the insertion of an additional codon in KOS relative to F, a 19-amino-acid sequence in F (18 in KOS) where the sequences differ due to a shift of relative reading frame, and 9 other amino acid substitutions (Fig. 2). The amino acid substitutions do not make significant alterations in our predicted structure for the membrane orientation and secondary structure of gB, except for the presence of a Thr at amino acid 283 in place of the Ser seen in F. This is the same substitution seen in two of the mutants sequenced (R126/S8 and R126/B1). The significance of this difference is unclear at this time. Our structural prediction based on the sequence, however, differs considerably from that of Bzik et al. (10). The specific areas of difference are as follows.

(i) Our prediction of a 29-amino-acid signal sequence with the predicted cleavage occurring after Ala_{-3}-Ser_{-2}-Ala_{-1} is based on several well-defined properties of signal sequences (44, 60). This is in contrast to the 41-amino-acid sequence predicted by Bzik et al. (10) to serve as the signal sequence because it is hydrophobic.

(ii) The prediction of a 44-amino-acid hydrophobic sequence serving as a membrane-spanning anchor (10) is unlikely inasmuch as such a structure would be predicted either to pass through the membrane twice as a hairpin of two 22-amino-acid segments, thus placing the carboxy-terminal 109 amino acids of the protein on the exterior of the infected cell or virion, or to adopt a heretofore unheard of conformation in the membrane. Our prediction of three membrane-spanning segments is based on the presence of three hydrophobic peaks in a hydropathic analysis and structural predictions within each segment which are compatible with a model of three interacting membrane-spanning helices.

(iii) An important component of our model and a subject not discussed elsewhere is the exquisite conformational sensitivity of gB, as demonstrated in the gradient of reactivity of monoclonal antibody mutants in various immunoassays as a function of the denaturing ability of the assay environment (30). The requirement for and consequences of such conformational sensitivity remain objects of future investigation.

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