JOURNAL OF VIROLOGY, Nov. 1984, p. 431–435
0022-538X/84/110431-05$02.00/0

Vol. 52, No. 2

Fine Structure Analysis of Type-Specific and Type-Common Antigenic Sites of Herpes Simplex Virus Glycoprotein D

BERNHARD DIETZSCHOLD,[1]* ROSELYN J. EISENBERG,[2,3] MANUEL PONCE DE LEON,[3,4] ELLIS GOLUB,[3,5] FERENC HUDECZ,[1] ANGELA VARRICHIO,[1] AND GARY H. COHEN[3,4]

The Wistar Institute of Anatomy and Biology,[1] Department of Pathobiology, School of Veterinary Medicine,[2] Departments of Microbiology[4] and Biochemistry,[5] and Center for Oral Health Research, School of Dental Medicine,[3] University of Pennsylvania, Philadelphia, Pennsylvania 19104

Received 21 May 1984/Accepted 24 July 1984

The fine structure of the antigenic determinants of herpes simplex virus type 1 and 2 glycoprotein D (gD) was analyzed to determine whether structural differences underlie the differential immunogenicity of these glycoproteins. A region common to herpes simplex virus type 1 and 2 gD (amino acid residues 11 to 19) and two sites specific for herpes simplex virus type 2 gD (one determined by proline at position 7, the other determined by asparagine at position 21) were localized within the N-terminal 23 amino acids of gD by synthesis of peptides and comparison of their cross-reactivity with antisera raised to herpes simplex virus type 1 and 2 gD. The secondary structure of these peptides, as predicted by computer analysis, is discussed in relation to their immunogenicity.

Glycoprotein D (gD) of herpes simplex virus (HSV) is one of four well-defined and distinct glycoprotein components of the virion envelope (1; P. G. Spear, *in* B. Roizman, ed., *The Herpesviruses*, vol. 3, in press). Studies have shown that the protein is structurally and antigenically similar, although not identical, in the two serotypes of HSV (HSV type 1 [HSV-1] and HSV type 2 [HSV-2]) (1, 7, 8, 16). In previous studies with a panel of monoclonal antibodies, eight epitopes were mapped on gD (6); some of these are specific for one HSV serotype (gD-1 or gD-2), and others are common to both gD-1 and gD-2. The type-specific determinants reflect differences in structure between gD-1 and gD-2 that are ultimately related to differences in the amino acid sequence (2, 11, 17). Group VII monoclonal antibodies react with both gD-1 and gD-2, exhibit type-common virus-neutralizing activity, and recognize the reduced and alkylated form of gD, suggesting that the type-common epitope is sequential in nature. These antibodies also recognize a synthetic peptide corresponding to residues 8 to 23 of the mature form of gD-1 (4).

A comparison of the sequences of gD-1 (18) and gD-2 (11, 17) (strain 333; W. Wilcox, R. J. Eisenberg, and G. H. Cohen, unpublished data) revealed differences in two residues within the first 23 amino acids; alanine (A) at position 7 and aspartic acid (D) at position 21 in gD-1 correspond, respectively, to proline (P) at position 7 and asparagine (N) at position 21 in gD-2. As antisera raised to gD-2 failed to react with the original gD-1 synthetic peptide, we investigated whether the specific amino acids at positions 7 and 21 account for the differential antigenicity and immunogenicity of these glycoproteins. In the present study, we synthesized a series of overlapping peptides, corresponding to portions of the first 23 amino acids of gD-1, gD-2, or a combination of both, and mapped the fine structure of the antigenic region. Three epitopes in the first 23 amino acids of gD could be delineated; two of them were gD-2 specific, and one was type common. The possibility that amino acid differences effect changes in the gD secondary structure, resulting in differences in antigenic activity, is discussed.

* Corresponding author.

MATERIALS AND METHODS

Preparation of antisera and monoclonal antibodies. Anti-gD-1 and anti-gD-2 sera were prepared in rabbits against purified preparations of gD-1 and gD-2, respectively (5, 8). The preparation of group VII monoclonal antibodies (represented by antibody 170) with HSV-2 strain G has been described previously (6, 14). Antisera to the synthetic peptides were prepared by immunization of female New Zealand rabbits intramuscularly at five 1-week intervals with peptides, each covalently bound to keyhole limpet hemocyanin (12) and emulsified in 50% complete Freund adjuvant.

Peptide synthesis. Peptides were synthesized as previously described (4). (See Table 1 for a list of the synthesized peptides.)

Preparation of gD. The purification of gD from HSV-1 (strain HF)-infected KB cells and HSV-2 (strain SAVAGE)-infected BHK cells was carried out as previously described (8).

Immunoblot analysis. Immunoblot analysis of synthetic peptides was done as previously described (4).

Citraconylation of peptides. The reaction of peptides with citraconic anhydride was effected essentially as described by Habeeb and Atassi (9). Peptides (50 mg) were dissolved in 5 ml of H_2O, and the pH was adjusted to 8.5 with 5 N NaOH. At 30-min intervals for 4 h, 10-μl aliquots of citraconic anhydride were added. After the eighth addition of citraconic anhydride, the peptide mixture was stirred for 2 h at room temperature, dialyzed against H_2O, adjusted to pH 8.5 with NH_4OH, and lyophilized. To remove the blocking group, we dissolved the citraconylated peptides in 0.05 M sodium acetate (pH 4.2) and incubated the mixture for 3 h at 4°C.

Synthetic peptide secondary structures. The secondary structures of 1–23[1], 1–23[H], and 1–23[2] were predicted by use of the rules established by Chou and Fasman (2, 3).

RESULTS

Peptide sequence selection. Chemical and immunological information obtained from proteolytic cleavage fragments of gD, together with a computer prediction of the secondary

structure and hydrophilicity of gD (2, 3, 10), localized a major antigenic domain of gD (4). To further map antigenic epitopes within this domain which are recognized by type-common and type-specific antibodies, we synthesized a series of overlapping peptides ranging in length from 7 to 23 amino acids and corresponding to amino acid residues 1 to 23 of gD-1 or gD-2 (Table 1). HSV-1-specific peptides were synthesized with A at position 7 and D at position 21; HSV-2-specific peptides contained P and N at positions 7 and 21, respectively. 1–23[H] and 3–23[H] were synthesized to represent hybrids having the HSV-2-specific residue (P) at position 7 and the HSV-1-specific residue (D) at position 21.

Binding properties of synthetic peptides. The synthetic peptides were tested for their binding activity with gD-specific polyclonal and monoclonal antibodies. An immunoblot assay (4) with different antigens and antisera was used (Fig. 1 and Table 2). Rabbit anti-gD-1 hyperimmune sera (Fig. 1, rows 1 and 2) reacted with both gD-1- and gD-2-specific peptides ranging in length from 23 amino acids to 11 amino acids (lanes d–g and k–n). N-terminal 1–16[1] and 1–16[2] were not recognized (lanes h and i), nor were the two shortest C-terminal peptides, 17–23[1] and 17–23[2] (lanes c and j), except for a weak response with one antiserum (row 2, lane c). Thus, anti-gD-1 serum did not distinguish between D and N in position 21 and did not detect any determinant in 1–16[1] or 1–16[2].

Rabbit anti-gD-2 hyperimmune sera (Fig. 1, rows 3 and 4) did not react with HSV-1-specific peptides (lanes c–e, h, and n) but did react with HSV-2-specific peptides (lanes i and k–m) and 3–23[H] (lane f) and 1–23[H] (lane g), which contain the gD-2-specific P at position 7. Moreover, anti-gD-2 sera recognized 1–16[2] (lane i) but not 1–16[1] (lane h), in contrast with anti-gD-1 sera, which recognized neither. Anti-gD-2 sera also reacted with 8–23[2] (lane l) and 13–23[2] (lane k) (presumably detecting the N at position 21) but not with 8–23[1] (lane e) or 12–23[1] (lane d). Anti-gD-2 sera did not react with the smallest molecule, 17–23[2] (lane j). Monoclonal antibody 170 reacted in a type-common fashion with gD-1-, gD-2-, and both gD-1- and gD-2-specific peptides of residues 8 to 23, 3 to 23, and 1 to 23 (row 5, lanes e–g and l–n). A slight reaction was also seen with 13–23[2] (lane k).

As shown in Fig. 1 and indicated in Table 2, monoclonal antibody 170 reacts better with peptides than with native glycoprotein. This is probably because of the relatively low molar concentration of the 170 epitope of the intact molecule, as used in the assay, as compared with the higher molar concentration of the peptide.

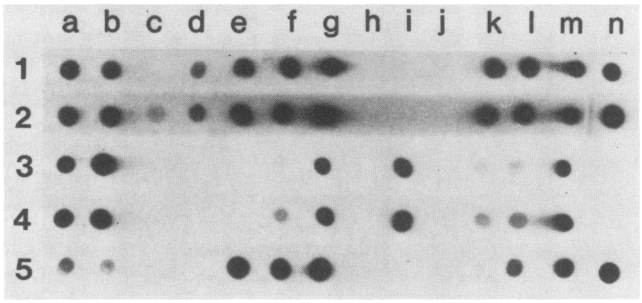

FIG. 1. Dot blot analysis of synthetic peptides with antibodies to gD. Antibodies: rows 1 and 2, anti-gD-1 (rabbits 1 and 2, respectively); rows 3 and 4, anti-gD-2 (rabbits 3 and 4, respectively); and row 5, monoclonal antibody 170. Antigens: lane a, gD-1 (28 ng); lane b, gD-2 (32 ng); lane c, 17–23[1] (500 ng); lane d, 12–23[1] (500 ng); lane e, 8–23[1] (500 ng); lane f, 3–23[H] (500 ng); lane g, 1–23[H] (500 ng); lane h, 1–16[1] (500 ng); lane i, 1–16[2] (500 ng); lane j, 17–23[2] (500 ng); lane k, 13–23[2] (500 ng); lane l, 8–23[2] (500 ng); lane m, 1–23[2] (500 ng); and lane n, 1–23[1] (500 ng).

Citraconylation. The reactivity of anti-gD-2 sera with 3–23[H] (Fig. 1, lane f) was lower than with 1–23[H] (Fig. 1, lane g), suggesting that the first two N-terminal amino acids are important. Citraconylation of the lysine residues (at positions 1, 10, and 20) in 1–16[2] and 1–23[H] abolished antigenic activity (Fig. 2A, row 2, lane h, and Fig. 2B, lane b). Removal of this blocking group restored activity (Fig. 2A, lane i, and Fig. 2B, lane c). Thus, one or both of the two lysines at positions 1 and 10 in these peptides are involved in antigenic activity.

In contrast, citraconylation of 1–23[H] had no effect on the reactivity of anti-gD-1 serum (Fig. 2A, row 1) or of monoclonal antibody 170 (data not shown) with this peptide. Therefore, lysine residues at positions 1, 10, and 20 are not involved in the process of recognition by these antibodies.

With respect to their reactivity with glycoproteins, only antisera raised against 8–23[1] exhibited both low binding activity (Fig. 3, row 5, lanes a and b) and low virus-neutralizing activity (4). The binding and neutralizing activities of anti-8–23[1] serum were comparable to those of monoclonal antibody 170 (Fig. 1, row 5, lanes a and b) (4).

TABLE 1. Sequences of synthetic peptides mapped within the first 23 amino acids of HSV gD

Peptide[a]	HSV type specificity	Amino acid sequence[b]
1–23[1]	1	K Y A L A D **A** S L K M A D P N R F R G K **D** L P
1–23[2]	2	K Y A L A D **P** S L K M A D P N R F R G K **N** L P
1–23[H]	1 and 2	K Y A L A D **P** S L K M A D P N R F R G K **D** L P
1–16[1]	1	K Y A L A D **A** S L K M A D P N R
1–16[2]	2	K Y A L A D **P** S L K M A D P N R
3–23[H]	1 and 2	A L A D **P** S L K M A D P N R F R G K **D** L P
8–23[1]	1	S L K M A D P N R F R G K **D** L P
8–23[2]	2	S L K M A D P N R F R G K **N** L P
12–23[1]	1	M A D P N R F R G K **D** L P
13–23[2]	2	A D P N R F R G K **N** L P
17–23[1]	1	R F R G K **D** L P
17–23[2]	2	R F R G K **N** L P

[a] Ranges (e.g., 1–23) indicate amino acid residues, numbers in brackets indicate the HSV type that the peptide was specific for, and H (for hybrid) in brackets indicates that the peptide contained the HSV-2-specific residue (P) at position 7 and the HSV-1-specific residue (D) at position 21.

[b] A, Alanine; D, asparagine; F, phenylalanine; G, glycine; K, lysine; L, leucine; M, methionine; N, asparagine; P, proline; R, arginine; S, serine; and T, tyrosine. Boxes indicate differences in positions 7 and 21.

TABLE 2. Reactivity of anti-gD and antipeptide sera with the corresponding antigens

Anti-serum	Reactivity[a] with indicated antigen													
	gD-1	gD-2	17–23[1]	17–23[2]	12–23[1]	13–23[2]	8–23[1]	8–23[2]	3–23[H]	1–16[1]	1–16[2]	1–23[H]	1–23[1]	1–23[2]
gD-1	+++	+++	+	−	+++	+++	+++	+++	+++	−	−	+++	+++	+++
gD-2	+++	+++	−	−	−	+	−	+	+	−	+++	+	−	+++
170	+	+	−	−	−	+	+++	++	+++	−	−	+	+++	+++
1–16[1]	−	−	NT	NT	NT	NT	+++	+++	NT	+++	+++	NT	NT	NT
1–16[2]	−	−	NT	NT	NT	NT	+++	+++	NT	+++	+++	NT	NT	NT
8–23[1]	+	+	NT	NT	NT	NT	+++	+++	NT	++	++	NT	NT	NT
8–23[2]	−	−	NT	NT	NT	NT	−	+++	NT	++	++	NT	NT	NT

[a] +++, Very good; ++, good; +, weak; −, none; and NT, not tested.

Immunogenicity. To determine whether the synthetic peptides mimic the immunogenic properties of the entire protein, we coupled peptides of residues 1 to 16 and 8 to 23, both having gD-1- or gD-2-specific sequences, to keyhole limpet hemocyanin by using a C-terminal cysteine (12) and evaluated their immunogenicity after injecting them into rabbits. These peptides were also chosen because they bear the HSV-1- or HSV-2-specific sites, as shown by their reactivities with gD-1 or gD-2 hyperimmune serum, respectively. The reactivities of the antipeptide sera were tested in an immunoblot assay (Fig. 3). Antisera raised to 1–16[1] (row 3) and 1–16[2] (row 4) reacted with both of the corresponding peptides (lanes d and e) and did not distinguish the amino acid change at position 7 in the peptide. These sera are unlike the antisera to either purified gD-1 or purified gD-2 (rows 1 and 2), indicating that the synthetic peptides probably assumed a conformation different from that of the native protein. In contrast, antisera raised to the HSV-2-specific peptide 8–23[2] (row 6) only recognized peptides having the HSV-2-specific N at position 21 (lane g); antisera raised against 8–23[1] (row 5) recognized peptides having either N at position 21 (lane g) or D at position 21 (lane f). Thus, anti-8–23[2] and anti-8–23[1] sera mimicked the reactivity of anti-gD-2 and anti-gD-1 sera with the same peptides (cf. rows 1 and 2 with rows 5 and 6). In addition, the antipeptide sera exhibited some cross-reactivity with other overlapping peptides. For example, anti-1–16[1] reacted with 8–23[2] (row 3, lane g) but reacted only slightly with 8–23[1] (row 3, lane f). Anti-1–16[2] reacted slightly with 8–23[2] (row 4, lane g) but did not react with 8–23[1] (row 4, lane f).

Synthetic peptide secondary structures. To further analyze the differences in the antigenicity and binding properties of the synthetic peptides resulting from different amino acids at positions 7 and 21, we examined the effects of these amino acid changes on the predicted secondary structures of the corresponding peptides. The secondary structures of 1–23[1], 1–23[H], and 1–23[2], predicted as described above, are shown in Fig. 4. This analysis shows that 1–23[1] consists of an α helical structure at the N terminus followed by one β turn (Fig. 4A). When the A in position 7 is substituted for by a P, the α helical structure at the N terminus is abolished and an additional β turn is predicted (1–23[H]) (Fig. 4B). 1–23[2], with N at position 21, was predicted to have an additional β turn at the C-terminal end, yielding a structure consisting of three β turns (Fig. 4C).

DISCUSSION

The results of these studies indicate the presence of two regions of gD within amino acids 1 to 23 capable of binding type-specific antibodies, although there are no discrete boundaries between these two regions. One of these (region 1) is contained within the first 16 amino acids of gD-2 and

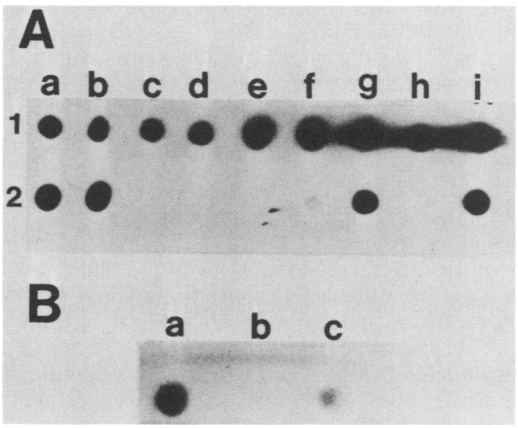

FIG. 2. Dot blot analysis of citraconylated synthetic peptides. (A) Row 1, anti-gD-1; row 2, anti-gD-2; lane a, gD-1; lane b, gD-2; lane c, 17–23[1]; lane d, 13–23[1]; lane e, 8–23[1]; lane f, 3–23[H]; lane g, 1–23[H]; lane h, 1–23[H] treated with citraconic anhydride; and lane i, 1–23[H] treated with citraconic anhydride and then decitraconylated. (B) Anti-gD-2 serum reacted against: lane a, 1–16[2]; lane b, 1–16[2] treated with citraconic anhydride; and lane c, 1–16[2] treated with citraconic anhydride and then decitraconylated.

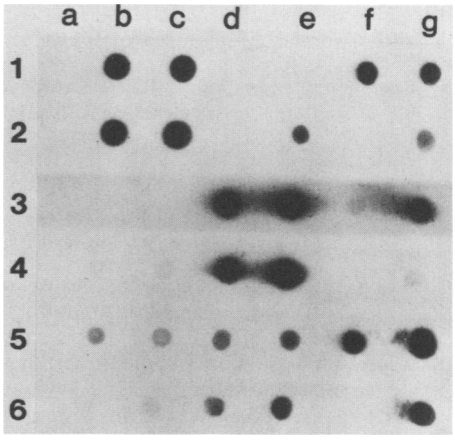

FIG. 3. Dot blot analysis of antisera from rabbits immunized with synthetic peptides bound to keyhold limpet hemocyanin. Antibodies: row 1, anti-gD-1; row 2, anti-gD-2; row 3, anti-1–16[1]; row 4, anti-1–16[2]; row 5, anti-8–23[1]; and row 6, anti-8–23[2]. Antigens: lane a, KB cell extract; lane b, gD-1 (28 ng); lane c, gD-2 (32 ng); lane d, 1–16[1] (500 ng); lane e, 1–16[2] (500 ng); lane f, 8–23[1] (500 ng); and lane g, 8–23[2] (500 ng).

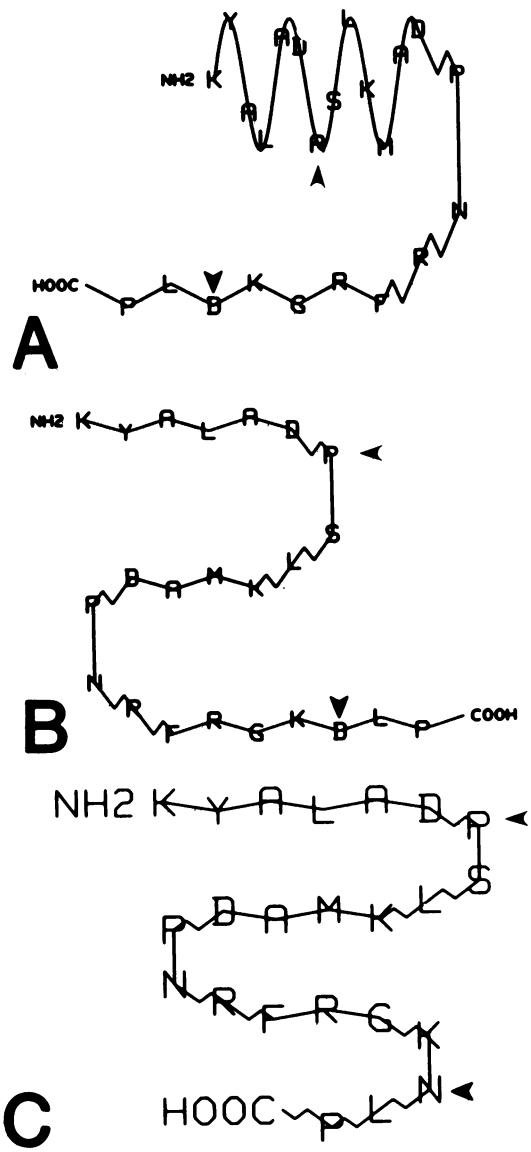

FIG. 4. Predicted secondary structures of 1–23[1] (A), 1–23[H] (B), and 1–23[2] (C). The secondary structures of the peptides was predicted by a computer program with the rules devised by Chou and Fasman (2). Probabilities for occurrence with a helix (Pa), a pleated sheet (Pb), and β turns (Pt) were evaluated with stringent conditions: (Pt > 1.0×10^{-4} or Pt > 7.5×10^{-5}, Pt > Pa and Pt > Pb). Arrows indicate positions 7 and 21. See Table 1, footnote *a*, for definitions of letters.

appears to involve one or both of the lysines at positions 1 and 10 and P at position 7. The same peptide with A at position 7 is not recognized by anti-gD-1 or anti-gD-2 sera, possibly because this sequence is buried within the intact glycoprotein. The second region (region 2), which denotes HSV-2 specificity, is located closer to the carboxy end of the 23-amino acid sequence, as anti-gD-2 sera reacted with peptides containing residues 8 to 23 or 12 to 23, but only when residue 21 was N. Antibodies to the amino terminus of gD-1 are produced predominantly against region 2. However, it is difficult to assess whether there is gD-1 specificity in region 2, as anti-gD-1 sera reacted with both gD-1 and gD-2 peptides of residues 8 to 23, 12 to 23, and 13 to 23. In contrast to region 1 of gD-1, which does not seem to induce significant antibody formation, region 1 of gD-2 is highly immunogenic and induces type-specific antibodies at the expense of the gD-2 region, which induces only weakly reactive HSV-2-specific antibodies. The enhanced immunogenicity of region 1 of gD-2 is probably due to the β turn caused by the P substitution at position 7.

Monoclonal antibody 170, a group VII antibody, binds to 8–23[1] and 8–23[2], suggesting that the epitope recognized by group VII antibodies is unaffected by the difference in the amino acid at position 21. As the antibody also reacted with citraconylated 1–23[H], it is quite likely that the group VII epitope does not involve the two lysines located at positions 10 and 20. This epitope is probably located between residues 11 and 19, where the residues are identical in gD-1 and gD-2 and are predicted to assume similar secondary structures. Thus, the group VII epitope appears to be truly type common and represents a third antigenic region in the first 23 N-terminal amino acids of gD.

In the intact protein, a difference at position 7 had a profound effect on the immune response, as measured by the difference in the reactivity of anti-gD sera with 1–16[1] and 1–16[2]; however, antisera prepared against these peptides failed to discriminate between 1–16[1] and 1–16[2], suggesting that these peptides probably assumed less distinct structures in solution than those assumed by these peptides in the intact protein. In contrast, antisera prepared against the peptides of residues 8 to 23 mimicked the pattern of reactivity of the corresponding anti-gD sera to the intact glycoproteins, i.e., anti-8–23[2] serum reacted with 8–23[2] but not with 8–23[1], whereas anti-8–23[1] serum reacted with both 8–23[1] and 8–23[2]. The unique reactivity of anti-8–23[2] serum might result from 8–23[2] adopting the native conformation found in the intact gD molecule. Alternatively, the structure might be stabilized by coupling to keyhold limpet hemocyanin. As the computer analysis (Fig. 4C) predicts that the change from D to N results in an additional β turn near the C terminus, it may be this structure that is specifically recognized by anti-8–23[2] serum. Anti-8–23[1] serum probably recognizes a less ordered structure. As peptides in solution may not adopt a single stable structure (13), 8–23[2] and 8–23[1] might exist in similar conformations in solution, and this could explain why anti-8–23[1] serum recognized both 8–23[1] and 8–23[2].

With respect to the intact glycoprotein, regions 1 and 2 appear to be highly immunogenic. On the other hand, antisera prepared against synthetic peptides containing one of the two antigenic regions exhibited no binding activity (anti-1–16[1], anti-1–16[2], and anti-8–23[2]) or weak binding activity (anti-8–23[1]) with the native glycoprotein, indicating that regions 1 and 2 as presented by the synthetic peptides differ from those found in the intact gD molecule. The poor reactivity of antipeptide sera might rest in the greater conformational freedom of peptides in solution, thereby reducing the antibody response to a predominant structure which may exist in the native glycoprotein. The immunogenicity of the synthetic peptide containing both regions was increased (data not shown), suggesting that the larger peptide introduced, like the native molecule, greater conformational constraints. The longer peptides also conferred good protection against challenge infection with HSV, and antisera produced against these peptides exhibited high titers of neutralizing antibody (unpublished data).

The close correspondence between secondary structure and immunological properties as observed in these studies suggest that modifications in both the length and sequence of

the antigenic peptide, affecting secondary structure, might prove useful in enhancing immunogenicity. The importance of this correlation has been recently demonstrated with synthetic cytochrome *c* peptides, which have T-cell stimulatory activity (15).

ACKNOWLEDGMENTS

We thank Denise Galloway for providing the clone pDG310, which contains the gene for gD-2 from HSV-2 strain 333 used by R.J.E. and G.H.C. for sequencing. We also thank Lenore Pereira for providing monoclonal antibody 170 and Marina Hoffman for carefully reading the manuscript.

This work was supported by Public Health Service grant DE-02623 from the National Institute of Dental Research, Public Health Service grants AI-18289 and AI-09706 from the National Institute of Allergy and Infectious Diseases, and a grant from American Cyanamid Co.

LITERATURE CITED

1. **Balachandran, N., D. Harnish, W. E. Rawls, and S. Bacchetti.** 1982. Glycoproteins of herpes simplex virus type 2 as defined by monoclonal antibodies. J. Virol. **44**:344–355.
2. **Chou, P. Y., and G. D. Fasman.** 1974. Conformational parameters for amino acids in helical β-sheet and random coil regions. Biochemistry **13**:211–222.
3. **Chou, P. Y., and G. D. Fasman.** 1974. Prediction of protein conformation. Biochemistry **13**:222–245.
4. **Cohen, G. H., B. Dietzschold, M. Ponce de Leon, D. Long, E. Golub, A. Varrichio, L. Pereira, and R. J. Eisenberg.** 1984. Localization and synthesis of an antigenic determinant of herpes simplex virus glycoprotein D that stimulates the production of neutralizing antibody. J. Virol. **49**:102–108.
5. **Cohen, G. H., M. Katze, C. Hydrean-Stearn, 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.
6. **Eisenberg, R. J., D. Long, L. Pereira, B. Hampar, M. Zweig, and G. H. Cohen.** 1982. Effect of monoclonal antibodies on limited proteolysis of native glycoprotein gD of herpes simplex virus type 1. J. Virol. **41**:478–488.
7. **Eisenberg, R. J., M. Ponce de Leon, and G. H. Cohen.** 1980. Comparative structural analysis of glycoprotein gD of herpes simplex virus types 1 and 2. J. Virol. **35**:428–435.
8. **Eisenberg, R. J., M. Ponce de Leon, L. Pereira, D. Long, and G. H. Cohen.** 1982. Purification of glycoprotein gD of herpes simplex virus types 1 and 2 by use of monoclonal antibody. J. Virol. **41**:1099–1104.
9. **Habeeb, F. S. A., and M. Z. Atassi.** 1979. Enzymatic and immunochemical properties of lysozyme. Evaluation of several amino group reversible blocking reagents. Biochemistry **9**:4939–4944.
10. **Hopp, T. P., and K. R. Woods.** 1981. Prediction of protein antigenic determinants from amino acid sequences. Proc. Natl. Acad. Sci. U.S.A. **78**:3824–3828.
11. **Lasky, L. A., and D. Dowbenko.** 1984. DNA sequence analysis of the type-common glycoprotein-D genes of herpes simplex virus types 1 and 2. DNA (N.Y.) **3**:23–29.
12. **Liu, F. T., M. Zinnecker, T. Hamaoka, and D. H. Katz.** 1979. New procedures for preparation and isolation of conjugates of proteins and a synthetic copolymer of D-amino acids and immunochemical characterization of such conjugates. Biochemistry **18**:690–697.
13. **Niman, H. L., A. Houghton, L. E. Walker, R. A. Reisfeld, I. A. Wilson, J. M. Hogle, and R. A. Lerner.** 1982. Generation of protein-reactive antibodies by short peptides is an event of high frequency: implications for the structural basis of immune recognition. Proc. Natl. Acad. Sci. U.S.A. **80**:4949–4953.
14. **Pereira, L., D. V. Dondero, D. Gallo, V. Devlin, and J. D. Woodie.** 1982. Serological analysis of herpes simplex virus types 1 and 2 with monoclonal antibodies. Infect. Immun. **35**:363–367.
15. **Pincus, M. R., F. Gerewitz, R. M. Schwartz, and M. A. Scheraga.** 1983. Correlation between the conformation of cytochrome C peptides and their stimulating activity in a T lymphocyte proliferation assay. Proc. Natl. Acad. Sci. U.S.A. **80**:3297–3300.
16. **Showalter, S. D., M. Zweig, and B. Hampar.** 1981. Monoclonal antibodies to herpes simplex virus type 1 proteins, including the immediate-early protein ICP 4. Infect. Immun. **34**:684–692.
17. **Watson, R. J.** 1983. DNA sequence of the herpes simplex virus type 2 glycoprotein D gene. Gene **26**:307–312.
18. **Watson, R. J., J. H. Weis, J. S. Salstrom, and L. W. Enquist.** 1982. Herpes simplex type 1 glycoprotein D gene: nucleotide sequence and expression in *Escherichia coli*. Science **218**:381–383.