JOURNAL OF VIROLOGY, Feb. 1988, p. 454–462
0022-538X/88/020454-09$02.00/0

Vol. 62, No. 2

Mapping of Functional and Antigenic Domains of the α4 Protein of Herpes Simplex Virus 1

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Received 10 September 1987/Accepted 4 November 1987

Monoclonal antibodies to α4, the major regulatory protein of herpes simplex virus 1, have been shown to differ in their effects on the binding of the protein to its DNA-binding site in the promoter-regulatory domain of an α gene. To map the epitopes, we expressed truncated genes in transient expression systems. All 10 monoclonal antibodies tested reacted with the N-terminal 288-amino-acid polypeptide. To map the epitopes more precisely, 29 15-mer oligopeptides, overlapping by five amino acids at each end, were synthesized and reacted with the monoclonal antibodies. The nine reactive monoclonal antibodies were mapped to seven sites. Of the two monoclonal antibodies which blocked the binding of α4 to DNA, one (H950) reacted with oligopeptide no. 3 near the N terminal of the protein, whereas the second (H942) reacted with oligopeptide no. 23 near the C terminus of the 288-amino-acid polypeptide. In further tests, oligopeptide no. 19 was found to compete with two host proteins, designated as αH1 and αH2-αH3, for binding to DNA as well as to retard DNA in a band shift assay, whereas oligopeptides no. 26, 27, and 28 enhanced the binding of α4 to DNA. Moreover, oligopeptide no. 27 was also found to retard DNA in a band shift assay. Polypeptide no. 19 competed with α4 for binding to DNA, whereas no. 27 neither enhanced nor competed with the binding of the host polypeptide αH1 to its binding site in the promoter-regulatory domain of an α gene, but did enhance the binding of the αH2-αH3 protein to its binding site. In contrast to these results, the truncated α4 polypeptide, 825 amino acids long, bound to the viral DNA, whereas a shorter, 519-amino-acid-long, truncated polypeptide did not. The 825-amino-acid polypeptide was previously shown to induce in transient expression systems the expression of a late (γ_2) viral gene.

The genes encoded in the herpes simplex virus 1 (HSV-1) genome form several groups whose expression is coordinately regulated and sequentially ordered in a cascade fashion (13, 14, 35). The first group, designated as the α genes, is induced by a structural component of the virion (3, 4, 28) and is transcribed by a host RNA polymerase in the absence of de novo protein synthesis (14, 21, 30). The expression of the later groups requires functional α proteins, but especially the major *trans*-acting regulatory protein identified as the infected-cell protein no. 4 (ICP4) or α4 (7–12, 26, 29, 31, 33, 34).

The α4 protein is 1,298 amino acids long (23); the apparent molecular weight of the newly synthesized protein, based on its electrophoretic mobility in denaturing gels, is approximately 163,000 (25). The protein undergoes extensive post-translational modification; it is phosphorylated in infected cells (36) and can be poly(ADP)ribosylated in vitro (32). In denaturing one-dimensional gels it forms at least three bands differing in electrophoretic mobility (25). In two-dimensional separation systems, it forms at least 20 spots (1). In its native state it exists as a homodimer (24). Mapping studies of temperature-sensitive (*ts*) mutations in the α4 gene have suggested that the functional domain of the gene extends over a large portion of its coding domain (10, 33). More recent studies of deletion mutants have shown that the functions related to the transition from α genes to genes expressed later in infection map in the N-terminal two-thirds of the gene (22).

The initial analyses of the functional domains of the α4 gene centered on the observation that, in DNA band shift assays, 2 of 10 monoclonal antibodies tested blocked the binding of the protein to its DNA-binding site. To map the epitopes recognized by these antibodies, we initially tested all the monoclonal antibodies for their capacity to bind truncated α4 proteins and ultimately extended these assays to synthetic oligopeptides. We report that all 10 monoclonal antibodies reacted with epitopes contained in the N-terminal 288 amino acids of the protein, that the epitope of one of the blocking monoclonal antibodies maps near a cluster of oligopeptides that enhanced the binding of α4 to DNA, that α4 is competent to bind to DNA in the absence of other viral proteins, and that the C-terminal 450 amino acids of the α4 protein are not required for DNA-binding activity.

MATERIALS AND METHODS

Construction of recombinant plasmids. Plasmid pRB3611 contains the entire domain of the α4 gene extending 3′ from nucleotide −330 relative to the transcription initiation site of the gene to approximately 130 base pairs (bp) downstream from its polyadenylation site. The plasmid also contains a 410-bp *Hpa*II fragment containing the polyadenylation sequences of the thymidine kinase gene, engineered to contain stop codons in all possible reading frames and inserted in the correct transcriptional orientation 3′ to the α4 gene. Deletions of the 3′ end of the α4 gene, carried in pRB3611, were constructed by partial digestion of pRB3611 with *Hpa*II (pRB3824 and pRB3825) or *Nar*I (pRB3826 and pRB3827) and subsequent complete digestion with *Cla*I, which cleaves immediately 5′ to the thymidine kinase sequence in pRB3611. The digested plasmids were circularized with T4 DNA ligase and used to transform *Escherichia coli* to ampicillin resistance. Individual clones were screened by

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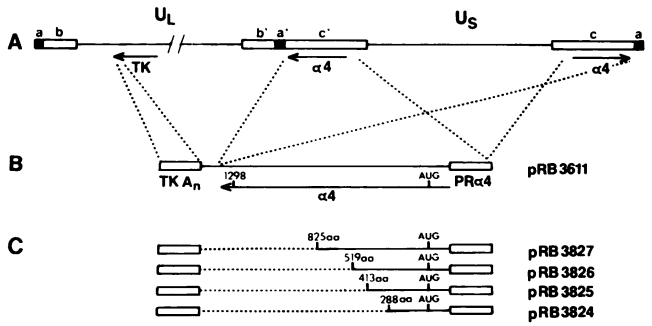

FIG. 1. Sequence map and location of deletion endpoints used in this study. (A) Location of both copies of the α4 gene and the single copy of the thymidine kinase gene in the prototype arrangement of the HSV-1 genome. (B) Map of pRB3611, which contains the entire α4 gene from HSV-1 (F) in addition to the polyadenylation sequence of the TK gene. (C) Contents of the deletion plasmids used in this study. The endpoint of each deletion is given in amino acids (aa) from the initiating methionine of the α4 gene at the end of the solid bar on each map. Each deletion results in the fusion of an internal portion of the α4 gene to a stop codon followed by a polyadenylation signal. Details of the construction of pRB3611 and its derivatives are given in Materials and Methods.

size on an agarose gel. The actual 3′ ends of clones pRB3827 and pRB3826 were verified by Maxam and Gilbert DNA sequencing, and the 3′ ends of clones pRB3824 and 3825 were estimated by restriction endonuclease site mapping. The amount of α4 coding sequence remaining in each of these constructs is shown in Fig. 1.

DNA transfections. DNA transfections were done as described by Kristie and Roizman (16) except that the 150-cm^2 flask cultures of 50% confluent baby hamster kidney (BHK) cells were each transfected with 64 μg of each plasmid DNA.

Preparation and analysis of protein samples. All samples were prepared from isolated nuclei 48 h posttransfection. For analyses of the reactivity of polypeptides with monoclonal antibodies, nuclei were isolated by centrifugation (10 min in an Eppendorf microfuge) of transfected BHK cells harvested in a solution consisting of 0.4% (vol/vol) Nonidet P-40, 0.25 M sucrose, 10 mM NaCl, 3 mM $MgCl_2$, 10 mM Tris hydrochloride (pH 7.7), and 25% (vol/vol) glycerol. Before electrophoresis in a 12% (wt/vol) denaturing polyacrylamide gel, the nuclei were sonicated and solubilized in disruption buffer as described elsewhere (2). The electrophoretically separated proteins were transferred electrically to a nitrocellulose sheet and reacted with monoclonal antibody as described elsewhere (2). The preparation of nuclear extracts from transfected BHK cells for DNA-binding assays was done as described elsewhere (17, 18), except that all centrifugation steps were done in an Eppendorf microfuge at 4°C.

DNA-binding assays. The conditions for the DNA-binding assays were as described elsewhere (17, 18). DNA-binding reaction buffer consisted of 20 mM Tris hydrochloride (pH 7.6), 50 mM KCl, 1 mM EDTA, 0.05% (vol/vol) Nonidet P-40, 5% (vol/vol) glycerol, and 50 μg of bovine serum albumin per ml. The probe was prepared by 5′-end labeling the 182-bp *Eco*RI-*Hin*dIII fragment carried in pRB3563 and containing the promoter of the α0 gene extending from +72 to −110 relative to its transcription initiation site (17). The reaction mixtures were electrophoretically separated in a 4.5% polyacrylamide gel, and the DNA-protein complexes were visualized by autoradiography.

Reactions that assayed the binding of peptide to DNA used the buffer and probe described above. In these reactions the concentration of peptide was 5 mM and the resulting oligopeptide-DNA complexes were separated on an 8% polyacrylamide gel.

The standard conditions for DNA-binding reactions that included synthetic oligopeptides were as follows. The DNA-binding mixture, consisting of 0.5 to 10 mM concentrations of each of the synthetic peptides, 0.5 μg of nuclear extract of infected HeLa cells, and 4 μg of sonicated salmon sperm DNA in the DNA-binding reaction buffer described above, was allowed to react for 15 min at room temperature. At that time, 20,000 cpm of the labeled α0 probe DNA was added and allowed to react for an additional 20 min at room temperature. The reaction mixtures were then electrophoretically separated on a 4% polyacrylamide gel, and the resulting DNA-protein complexes were visualized by autoradiography.

Reactions that assayed the effect of oligopeptides 19 and 27 on the formation of host complexes were done in exactly the same way as those using infected-cell extracts, except that 1 μg of nuclear extract from uninfected HeLa cells was substituted for infected HeLa cell nuclear extract and the reaction buffer contained 0.9 mg of bovine serum albumin per ml. These reactions, however, used as a probe the 48α27R fragment as described in the text.

Oligopeptide synthesis and ELISA. Synthetic oligopeptides were prepared as described by Houghten (15). Enzyme-linked immunosorbent assays (ELISAs) were done as described by Houghten except (i) radioimmunoassay-grade bovine serum albumin (1%, wt/vol; Sigma Chemical Co., St. Louis, Mo.) was used to block microtiter wells after adsorption of the oligopeptides, and (ii) primary antibodies were incubated with the oligopeptides at room temperature overnight.

Computer analysis of protein and oligopeptide sequences. Hydropathic analyses were done by the method of Kyte and Doolittle (20), and peptide secondary structure predictions were made using the method of Chou and Fasman (5) on a Hewlett-Packard 9000 computer.

RESULTS

Independently derived monoclonal antibodies to the α4 protein react with the N-terminal domain of the α4 protein. The initial purpose of these studies was twofold. The first goal was to select from a panel of 10 monoclonal antibodies those that react with the N-terminal domain of the α4 gene, to be able to identify the gene products of deletion mutants in the 3′ domain of the gene. The second objective was to take advantage of the observation that monoclonal antibodies to α4 differ with respect to their ability to block the binding of the α4 protein to the DNA, to define the functional domains of the α4 protein. In this series of experiments, five plasmids, one with an intact α4 gene and four with deletions in this gene, were constructed (Fig. 1). The plasmids were transfected into BHK cells. The lysates of cells harvested after 48 h were electrophoretically separated in a denaturing polyacrylamide gel, transferred to a nitrocellulose sheet, and reacted with the panel of 10 monoclonal antibodies. The results for 9 of the 10 monoclonal antibodies and truncated α4 proteins derived from plasmids pRB3824 and pRB3825 are shown in Fig. 2; one monoclonal antibody (H1091) was tested separately (data not shown). The significant finding was that each of the truncated α4 genes expressed polypeptides which reacted with all of the monoclonal antibodies. The smallest truncated gene, cloned as pRB3824 (Fig. 2C), formed several bands, possibly reflecting either premature termination of translation or degradation. The other trun-

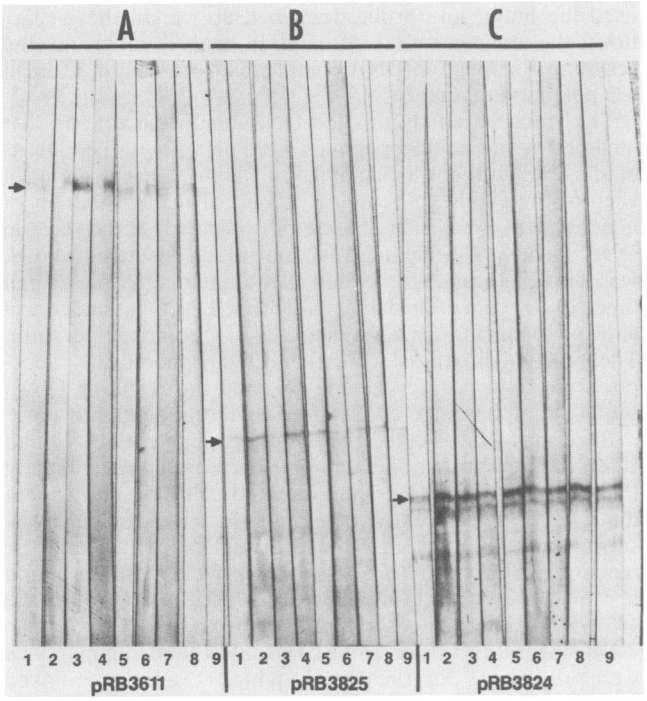

FIG. 2. Photographs of full-length and truncated α4 proteins electrophoretically separated in polyacrylamide gels, electrically transferred to nitrocellulose sheets, and reacted with monoclonal antibodies against α4 proteins. Panels A, B, and C contain nuclear proteins isolated from cells transfected with the plasmids pRB3611, pRB3825, and pRB3824, respectively. The size of the α4 protein expected from each plasmid is given in Fig. 1C. In each case nuclear proteins were prepared from cells 48 h posttransfection, electrophoretically separated in polyacrylamide gels, and transferred to nitrocellulose as described in Materials and Methods. The nitrocellulose sheets were cut into strips and reacted individually with monoclonal antibodies (1 through 9 at the bottom of each strip). The designations 1 through 9 refer to monoclonal antibodies H640, H953, H948, H942, H949, H950, H924, and H944, respectively.

1. met ala ser glu asn lys gln arg pro gly ser pro gly pro thr
2. ser pro gly pro thr asp gly pro pro pro thr pro ser pro asp
3. thr pro ser pro asp arg asp glu arg gly ala leu gly trp gly
4. ala leu gly trp gly ala glu thr glu glu gly gly asp asp pro
5. gly gly asp asp pro asp his asp pro asp his pro his asp leu
6. his pro his asp leu asp asp ala arg arg asp gly arg ala pro
7. asp gly arg ala pro ala ala gly thr asp ala gly glu asp ala
8. ala gly glu asp ala gly asp ala val ser pro arg gln leu ala
9. pro arg gln leu ala leu leu ala ser met val glu glu ala val
10. val glu glu ala val arg thr ile pro thr pro asp pro ala ala
11. pro asp pro ala ala ser pro pro arg thr pro ala phe arg ala
12. pro ala phe arg ala asp asp asp asp gly asp glu tyr asp asp
13. asp glu tyr asp asp ala ala asp ala ala gly asp arg ala pro
14. gly asp arg ala pro ala arg gly arg glu arg glu ala pro leu
15. arg glu ala pro leu arg gly ala tyr pro asp pro thr asp arg
16. asp pro thr asp arg leu ser pro arg pro pro ala gln pro pro
17. pro ala gln pro pro arg arg arg arg his gly arg trp arg pro
18. gly arg trp arg pro ser ala ser ser thr ser ser asp ser gly
19. ser ser asp ser gly ser ser ser ser ser ser ala ser ser ser
20. ser ala ser ser ser ser ser ser ser asp glu asp glu asp asp
21. glu asp glu asp asp asp gly asn asp ala ala asp his ala arg
22. ala asp his ala arg glu ala arg ala val gly arg gly pro ser
23. gly arg gly pro ser ser ala ala pro ala ala pro gly arg thr
24. ala pro gly arg thr pro pro pro pro gly pro pro pro leu ser
25. pro pro pro leu ser glu ala ala pro lys pro arg ala ala ala
26. pro arg ala ala ala arg thr pro ala ala ser ala gly arg ile
27. ser ala gly arg ile glu arg arg arg ala arg ala ala val ala
28. arg ala ala val ala gly arg asp ala thr gly arg phe thr ala
29. val ala gly arg asp ala thr gly arg phe thr ala gly gln pro

FIG. 3. Amino acid composition of the oligopeptides used in this study.

cated genes each specified a single polypeptide. The results indicate that the epitopes recognized by all of the monoclonal antibodies are located within the 288 amino acids located at the N terminus of the protein.

Mapping of the epitopes recognized by the monoclonal antibodies to the α4 protein. To map the epitopes recognized by the monoclonal antibodies, 29 oligopeptides covering the domain of the 288-amino-acid α4 polypeptide specified by the truncated gene contained in pRB3824 were synthesized. Each of the oligopeptides contained 15 amino acids and

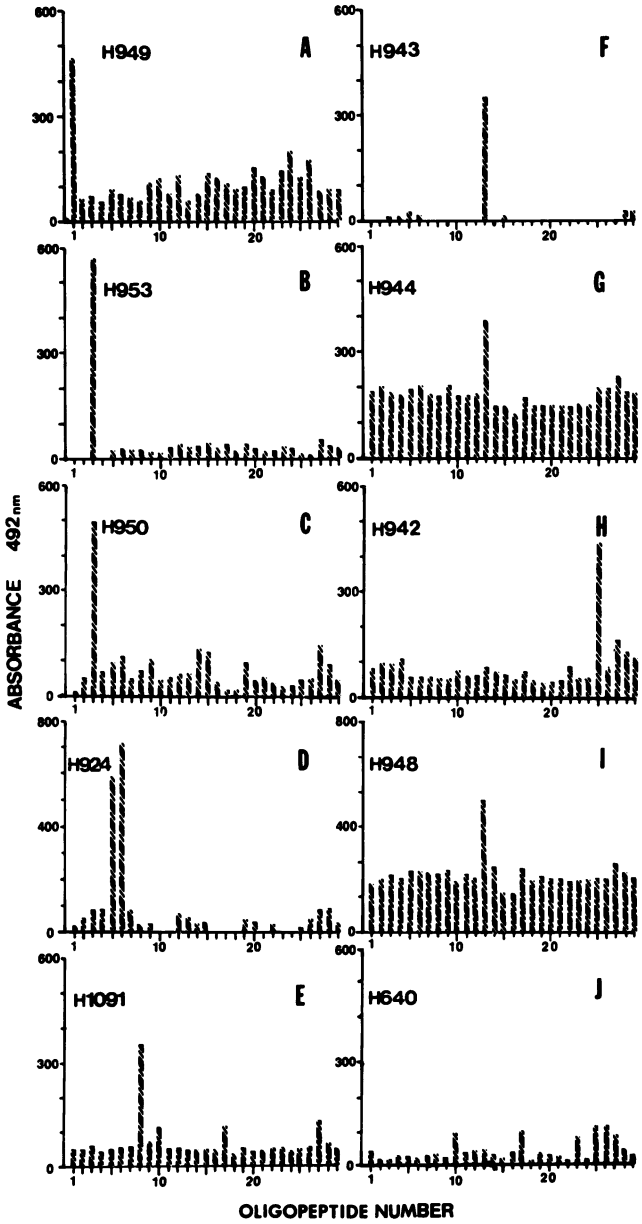

FIG. 4. Reactivity of monoclonal antibodies with synthetic polypeptides in ELISAs. Results of ELISAs of the oligopeptides shown in Fig. 3 with a panel of 10 independently derived monoclonal antibodies known to react with the α4 protein. The ELISAs are described in Materials and Methods. Each panel shows the reactivity of a monoclonal antibody with each of the oligopeptides, as measured by A_{492}, in an immunoperoxidase reaction using *o*-phenylenediamine as a substrate.

overlapped its adjacent peptides by 5 amino acids, as shown in Fig. 3 and 4B. The reaction of each monoclonal antibody with the 29 oligopeptides was assayed by ELISA as described by Houghten (15) and in Materials and Methods. The results of these assays (Fig. 4 and 5) were as follows. (i) Of the 10 monoclonal antibodies tested, only 1, H640, failed to react with any of the oligopeptides. The observation that this monoclonal antibody reacted with the truncated 288-amino-acid peptide but not with the oligopeptides suggests either that none of the oligopeptides contained the entire domain of the epitope or that the conformation assumed by the denatured 288-amino-acid polypeptide was different from those assumed by the individual oligopeptides. (ii) Two monoclonal antibodies, H950 and H953, reacted with oligopeptide no. 3. Inasmuch as H950 blocked the formation of the α4 protein-DNA complexes whereas H953 did not (17), it is likely that the epitopes of the two monoclonal antibodies occupy different sites on this oligopeptide. (iii) Monoclonal

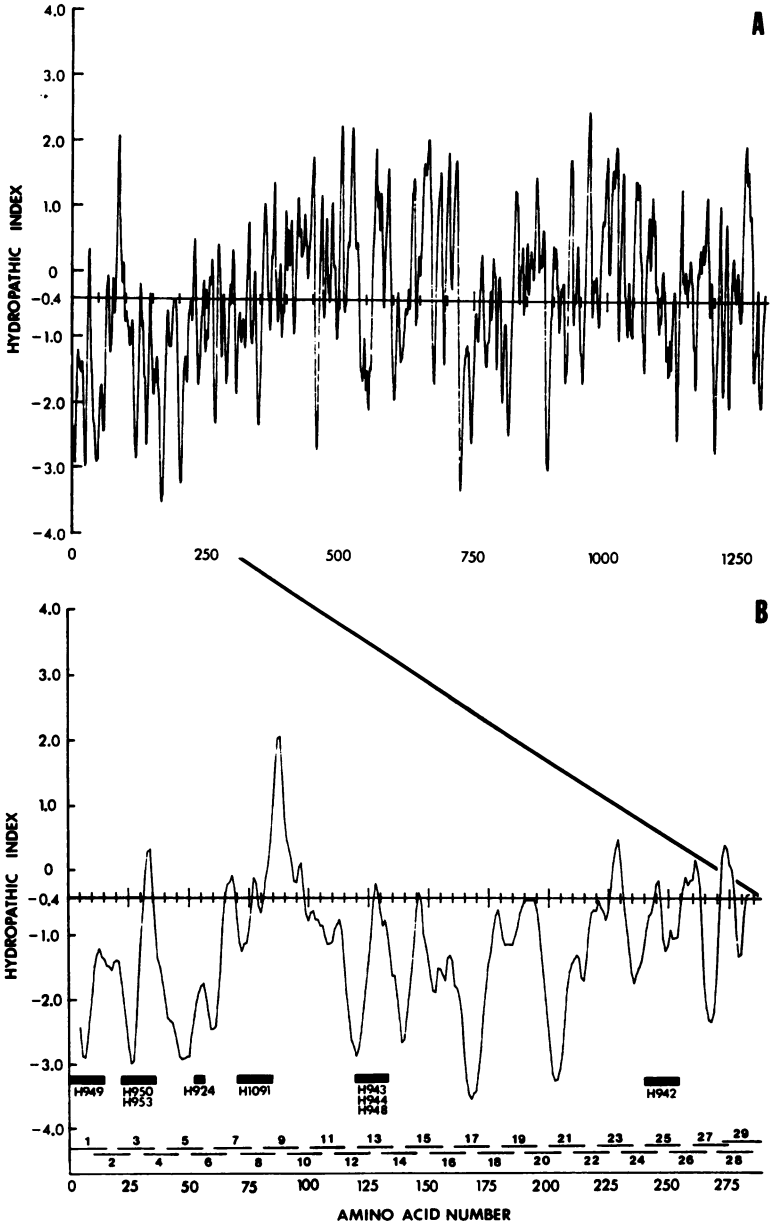

FIG. 5. Hydropathic analysis of the predicted α4 amino acid sequence and of the positions of the antigenic determinants recognized by the monoclonal antibodies used in this study. (A) Graphic representation of the hydropathic character of the entire α4 amino acid sequence as determined by the method of Kyte and Doolittle (20). (B) Expansion of panel A with particular reference to the N-terminal portion of the α4 protein. Both panels show the average hydropathicity values of incremental seven-amino-acid segments of the α4 protein, plotted as a function of their position in the α4 coding sequence. Panel B also contains the postions of the synthetic oligopeptides used in this study (thin bars) and their corresponding numerical designations. The amino acid compositions of the peptides used in this study and their numerical designations are given in Fig. 3. Also shown in panel B (thick bars) are the locations of the epitopes recognized by the monoclonal antibodies. Each bar is positioned over the oligopeptide to which a monoclonal antibody bound, and below each bar is a list of the antibody or antibodies that react with that oligopeptide.

antibodies H943, H944, and H948 each reacted with oligopeptide no. 13 and could not be differentiated with respect to their properties. (iv) H924 reacted with both peptides 5 and 6, suggesting that the epitope contains the shared amino acids. (v) Monoclonal antibody H942 reacted with oligopeptide no. 25. The epitope recognized by H942 is the most C terminal of all of those mapped, and like H950, H942 blocked the formation of the α4 protein-DNA complexes (18). (v) Hydropathic analyses (11) of the N-terminal stretch of 288 amino acids (Fig. 5) did not reveal a consistent hydrophilic property of the reacting oligopeptides. Most of the oligopeptides containing epitopic sites are average in hydrophilicity or encompass a domain that extends from a peak hydrophilic domain to a relatively hydrophobic domain (e.g., oligopeptides no. 13, 4-5, and 1). Two oligopeptides, no. 8 and 25, are on the average the most hydrophobic of those tested and found to be reactive.

The C terminus of α4 in DNA-binding activity. In a series of experiments to determine the role of the C terminus of α4 in DNA-binding activity, nuclear extracts from cells harvested 48 h posttransfection as described in Materials and Methods were reacted for 30 min with 40,000 cpm (0.2 ng) of 5′-end-labeled α0 promoter probe and subjected to electrophoresis on a 4% polyacrylamide gel as described elsewhere (17, 18). Previous studies have shown that the α4 protein specifically forms complexes with a specific sequence contained in this probe DNA (17). The results of the DNA-binding assay are shown in Fig. 6. Lanes 1 and 2 of Fig. 6 each contain an α4 protein-DNA complex (arrow) formed by the α4 protein synthesized from plasmids pRB3611 and pRB3827, respectively. Lanes 3 and 4, containing electrophoretically separated binding reactions formed by nuclear extracts derived from cells transfected with plasmids pRB3826 or pRB3825, did not show complexes that were not present in extracts made from untransfected cells (lane 5). These results indicate that the full-length α4 protein derived from transfection of pRB3611 is competent to bind to the α0 probe and demonstrated conclusively that no other viral factors need be present for α4 protein to bind to DNA. The DNA-protein complex formed in the presence of and specific for the nuclear extract of cells transfected with pRB3827 expressing the truncated α4 protein migrated faster than the complex formed by the intact α4 protein-DNA complex. The presence of the truncated α4 protein in these complexes could be deduced from the observation that monoclonal antibody H640 retarded the electrophoretic mobility of this complex as was previously shown for the intact α4 protein (17, 18). These observations suggest that the amino acids essential for the binding of α4 protein to its cognate site in the α0 DNA probe sequences are contained in the first 817 amino acids of the protein and, more specifically, between the C terminus of the largest fragment that did not bind (519 amino acids) and the amino acid 825 of the α4 protein.

Synthetic oligopeptides and the DNA-binding activity of α4. The purpose of the next series of experiments was to determine whether the synthetic peptides affect the binding of α4 to its cognate sites in the α0 promoter fragment. The labeled DNA probe used in this assay consisted of the α4-binding site in the promoter-regulatory domain of the α0 gene described in Materials and Methods. The standard conditions for all DNA-binding reactions that included synthetic oligopeptides are given in Materials and Methods. Nuclear extracts of infected HeLa cells served as a source of the α4 protein. The results were as follows.

(i) Initial experiments showed that only five oligopeptides, no. 17, 19, 26, 27, and 28, affected the binding of α4 protein to the α0 DNA probe or bound directly to the DNA in reaction mixtures containing 5 mM concentrations of individual oligopeptides (data not shown).

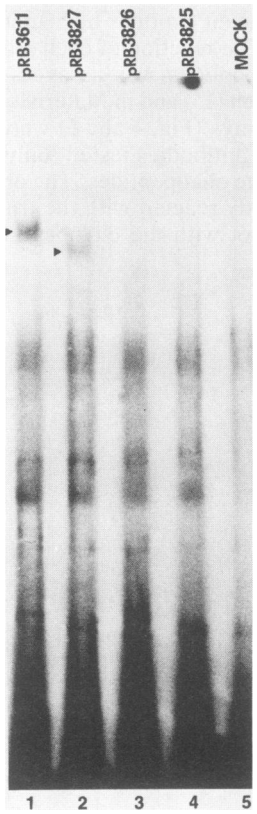

FIG. 6. Autoradiographic images of ^{32}P-labeled DNA-protein complexes electrophoretically separated in nondenaturing polyacrylamide gels. Nuclear extracts from BHK cells transfected with plasmids containing full-length and C-terminal-truncated α4 genes were reacted with a DNA fragment containing the α4 protein-binding domain of the promoter sequence of the α0 gene. Lanes 1 through 5, Electrophoretically separated DNA-protein complexes formed by nuclear extracts prepared from BHK cells transfected with the plasmids pRB3611, pRB3827, pRB3826, and pRB3825 and mock-transfected cells, respectively. Arrows in lanes 1 and 2 indicate the positions of the α4 protein-DNA complexes. Details of the transfections, preparation of nuclear extracts, DNA-binding reactions, and the preparation of the DNA probe are in Materials and Methods.

(ii) Oligopeptide no. 17 reproducibly appeared to precipitate the labeled DNA probe into complexes that failed to enter the polyacrylamide gel (data not shown).

(iii) Oligopeptide no. 19 blocked the binding of α4 to the labeled α0 DNA probe (Fig. 7A, lanes 6 through 9). The competition of the α0 DNA probe was oligopeptide concentration dependent and reduced the binding of α4 protein to the probe DNA by approximately 50% at the lowest concentration tested.

(iv) The partially overlapping oligopeptides no. 26, 27, and 28 enhanced the binding of α4 to the labeled α0 DNA probe (Fig. 7B and C). The enhancement was most pronounced in the case of oligopeptide no. 27 and least pronounced in the case of oligopeptide no. 28. In each instance, the extent of binding enhancement was oligopeptide concentration dependent.

Binding of oligopeptides no. 19 and 27 to the labeled α0 DNA probe. The ability of oligopeptides 19 and 27 either to

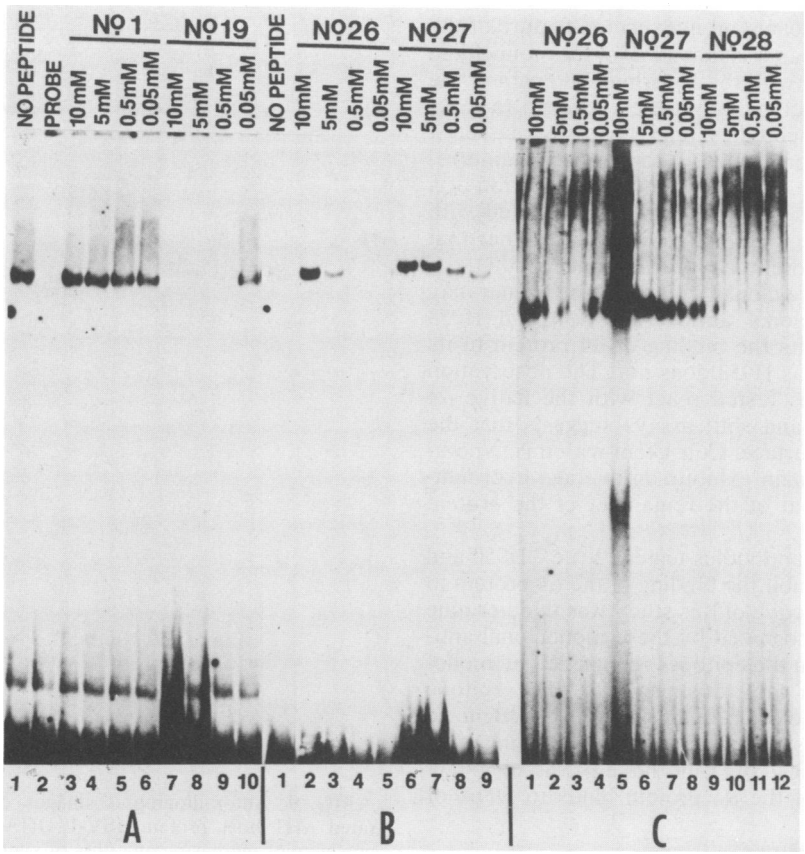

FIG. 7. Autoradiographic images of electrophoretically separated α4 protein-DNA complexes formed in the presence of oligopeptides no. 1, 19, 26, 27, and 28. Nuclear extracts of infected cells containing the α4 protein were reacted first with the oligopeptides and then with a DNA probe consisting of the ^{32}P-labeled DNA fragment containing the α4 protein-binding site of the promoter domain of the α0 gene as described in Materials and Methods. The preparation of the DNA probe, the reaction with the oligopeptides, and the conditions of electrophoretic separation were as described in Materials and Methods. (A) Lane 1, α4 protein-DNA complex formed in the absence of oligopeptides; lane 2, labeled DNA probe only; lanes 3 through 6, α4 protein-DNA complexes formed in the presence of oligopeptide no. 1 (this oligopeptide, like most oligopeptides, was found to be inert in the DNA-binding reaction); lanes 7 through 10, α4 protein-DNA complexes formed in the presence of various concentrations of oligopeptide no. 19. (B) Lane 1, α4 protein-DNA complexes formed in the absence of oligopeptides; lanes 2 through 5 and 6 through 9, α4 protein-DNA complexes formed in the presence of various concentrations of oligopeptides no. 26 and 27, respectively (these oligopeptides increase the amount of probe bound to α4 protein). (C) α4 protein-DNA complexes formed in the presence of oligopeptides no. 26 (lanes 1 through 4), 27 (lanes 4 through 8), and 28 (lanes 9 through 12). The amount of oligopeptide included in the DNA-binding reaction is given at the top of the corresponding lane.

compete with or to enhance the binding of the α4 protein to the α0 DNA probe raised the possibility that the synthetic oligopeptides were able to bind to the DNA probe. To test this hypothesis, each of the oligopeptides, at a concentration of 5 mM, was mixed with 0.1 ng of the labeled α0 DNA probe in binding reaction buffer containing increasing amounts of sonicated salmon sperm DNA. The mixtures were allowed to react for 30 min at room temperature and were then electrophoretically separated in an 8% polyacrylamide gel. The results (Fig. 8) showed that oligopeptides no. 19 and 27 were capable of retarding the electrophoretic mobility of the α0 probe DNA. Salmon sperm DNA competed with the probe DNA for the peptides at concentrations ranging from 5 μg for oligopeptide no. 19 to 5 ng for oligopeptide no. 27.

Specificity of the effects of oligopeptides no. 19 and 27 on the binding of proteins to DNA. To determine whether the effects of oligopeptides no. 19 and 27 on the binding of the α4 protein were specific, we tested the effects of these peptides on the binding of the host proteins αH1 and αH2-αH3 to their binding sites on the 48-bp fragment (48α27R) from the regulatory domain of the α27 gene (19). These proteins have been shown to bind to the *cis*-acting site of the α gene *trans*-inducing factor, a structural component shown to induce the α genes of HSV-1 (19). Furthermore, both αH1 and αH2-αH3 proteins can bind concurrently to DNA, forming a DNA-protein complex (αH1 + αH2-αH3) which migrates more slowly than either αH1 or αH2-αH3 protein-DNA complexes (T. M. Kristie and B. Roizman, manuscript submitted for publication). At concentrations of 0.5 and 5.0 mM, oligopeptide no. 19 competed with the αH1 and αH2-αH3 proteins for binding to DNA (Fig. 9). Oligopeptide no. 27 at the highest concentrations tested (5 mM) had no effect on the binding of αH1. However, the oligopeptide did enhance binding of the αH2-αH3 protein and the concurrent binding of both αH1 and αH2-αH3 proteins to the DNA fragment. Therefore it appears that the enhancement effect of oligopeptide no. 27 is not limited to the α4 protein and yet does not increase the affinity of all proteins for DNA, whereas oligopeptide no. 19 inhibited the formation of all complexes tested.

DISCUSSION

Epitopes recognized by the monoclonal antibodies against the α4 protein bind to the N-terminal 288-amino-acid domain

of the protein. The significant and unexpected feature of the results presented in this paper is that all 10 of the monoclonal antibodies tested reacted with the N-terminal domain of the protein. Of the 10 monoclonal antibodies, 1 failed to react with the synthetic oligopeptides, and the epitope that it recognizes may be discontinuous. The remaining nine reacted with the oligopeptides and identified the location of a minimum of six epitopes. Two oligopeptides reacted with more than one monoclonal antibody, suggesting either that the peptide contains more than one epitope or that the monoclonal antibodies are clonally related. The former may be the case for monoclonal antibodies H950 and H953 inasmuch as H950 inhibits the binding of α4 protein to its DNA-binding site whereas H953 does not. The observation that all of the antibodies tested react with the native α4 molecule in the DNA band shift assays suggests that the epitopic sites are on its surface. Consistent with this hypothesis, the N-terminal domain exhibits an average hydrophilicity far greater than that of the remainder of the protein (Fig. 5A).

Of the 10 monoclonal antibodies tested, 2 (i.e., H950 and H942) were shown to inhibit the binding of the α4 protein to its DNA, and major objective of this study was to determine whether the epitopes recognized by these monoclonal antibodies were distinct from the epitopes recognized by monoclonal antibodies which lack this property. The epitope recognized by H950 is in close proximity to those recognized by monoclonal antibodies that do not affect the binding of α4 protein to its DNA-binding site, and none of the oligopeptides from this region of the α4 protein binds to DNA or

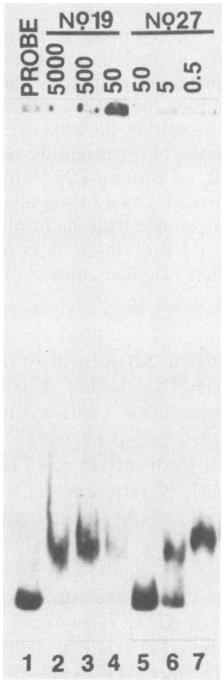

FIG. 8. Autoradiographic images of electrophoretically separated oligopeptide-DNA complexes formed by the reacting of synthetic oligopeptides no. 19 and 27 with the ^{32}P-labeled DNA fragment containing the α4 protein-binding site from the promoter domain of the α0 gene. Lanes 2 through 4 and lanes 5 and 6 contain 5-mM amounts of peptides no. 19 and 27, respectively, and increasing microgram amounts of competitor salmon sperm DNA as indicated at the top of each lane. Lane 1 contains only the α0 promoter DNA probe and indicates the position where unbound DNA migrates.

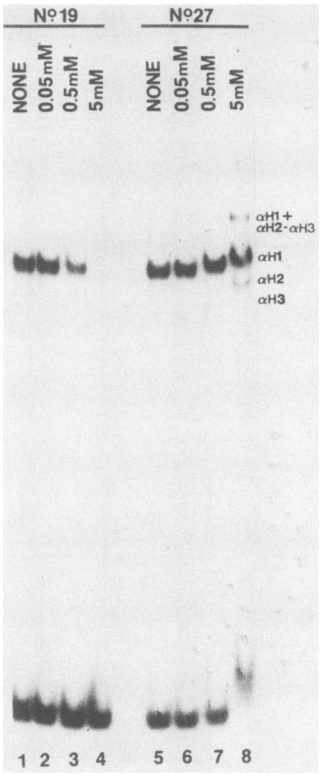

FIG. 9. Autoradiographic images of electrophoretically separated αH1 host protein-HSV-1 DNA complexes formed in the presence of increasing amounts of synthetic oligopeptide no. 19 or 27. The DNA probe was the 48-bp fragment from the α27 gene (48α27R DNA fragment) containing the *cis*-acting site for the α *trans*-inducing factor (19). Lanes 1 through 4 and 5 through 8 contain DNA-binding reactions to which various amounts of oligopeptides no. 19 and 27, respectively, were added. The DNA-binding reactions and electrophoretic separation in nondenaturing polyacrylamide gels were done as described in the text. The amount of oligopeptide added to the reaction mixture is indicated at the top of each lane.

interferes with the binding of α4 with the DNA probe. Conversely, the epitope recognized by H942 is located in close proximity to two oligopeptides, no. 19 and 27, which competed with or enhanced the binding of α4 to DNA, respectively, and which also bound to DNA. In this instance, H942 may block the binding reaction by sterically hindering the interaction of α4 with its DNA-binding site or the mutual interaction of different domains of the α4 protein required for binding to DNA.

Role of the amino acid sequences contained in oligopeptides no. 19 and 26 through 28 in the binding of α4 protein to its binding site. The salient features of the results were that oligopeptide no. 19 interfered with the binding of α4 protein to its DNA-binding site whereas oligopeptides no. 26 through 28 enhanced to binding. Studies of the competition of oligopeptide no. 19 with αH1 and αH2-αH3 proteins for their binding sites on DNA suggest that the inhibitory effect of this oligopeptide on the DNA-binding activity of α4 protein is not specific since the oligopeptide inhibits the DNA-binding activities of at least two other proteins, the host proteins αH1 and αH2-αH3.

The enhancement effect of oligopeptides no. 26 through 28 is less readily explainable. The unresolved questions are the specificity of binding and the sequence responsible for the

effect of oligopeptide no. 27. The former issue arises from the observation that oligopeptide no. 27 enhances α4 and αH2-αH3 but not αH1 protein binding to DNA, whereas the latter arises from the observation that oligopeptides no. 26, 27, and 28 as a group do not share the same amino acid sequence (there is none between no. 26 and no. 28) and yet have a similar effect on the binding of α4 protein to its DNA-binding site. Further studies on variants of the amino acid sequences contained in this region of the α4 protein may be necessary to resolve these questions and to determine whether the sequence plays a determinant role in the DNA-binding properties of the α4 protein.

It is of interest that analysis of the amino acid sequence of oligopeptides no. 19 and 27 by the method of Chou and Fasman (5) allows a prediction to be made about the secondary structure of these two oligopeptides and indicates that there is a high probability that these sequences would adopt ordered structures in the α4 protein. In the case of oligopeptide no. 19, the entire sequence is predicted to be involved in a succession of β turns with a turn probability product that is greater than 1.1×10^{-4} over the entire sequence, the minimum turn probability product for predicted β structures being 0.75×10^{-4} (5). This type of structure is atypical of DNA-binding motifs, but the sequence of oligopeptide 19 contains a predominance of serine residues which are capable of forming hydrogen bonds with guanine residues or the phosphate backbone of the DNA helix. Oligopeptide no. 27, on the other hand, is predicted to adopt a nine-amino-acid α-helical structure with a tetrapeptide α-helical propensity average of at least 1.11 over the length of the predicted helix, the minimum value for predicted α-helical structures being 1.03 (5). The structure of this helix is interesting in that it positions three of the four hydrophilic arginine residues on one side of the helix and the three hydrophobic alanine residues of the peptide on the other side. The predicted structure of this oligopeptide therefore exhibits a number of motifs that are present in the DNA-binding domains of other proteins such as the site-specific DNA-binding Cro protein from bacteriophage λ and the nonspecific DNA-binding protamines (27).

Functional and DNA-binding domains of the α4 protein. Previous studies have shown that the truncated α4 gene specifying an 825-amino-acid product was able to induce HSV-1 genes in a transient expression system (22), and in this study we have demonstrated that the truncated polypeptide is able to bind to viral DNA. Analyses of the phenotype of the truncated 825-amino-acid gene are incomplete, and the precise functions of the α4 gene encoded in the 3′ domain of the gene have not been identified. The observation that the sequence of the varicella-zoster virus analog of the α4 gene (6) contains several conserved domains of the α4 gene in the 3′ domain deleted from the truncated gene argues strongly for functions encoded in that domain which have not yet been identified.

Analyses of the functional domains within the 825-amino-acid α4 polypeptide by testing smaller variants of the α4 gene may be of very limited usefulness. For example, both the 825- and the 519-amino-acid polypeptides contain the same domains conserved in the varicella-zoster analog of the α4 gene, and both contain the site of rescue of the *ts*K mutation which blocks the expression of β and γ genes at the nonpermissive temperature (21).

The results obtained in this study do not prove that the binding site of the α4 protein is contained in the protein domain between amino acids 519 and 825, since there is a possibility that the conformation of the 519-amino-acid polypeptide is so altered from that of the 825-amino-acid polypeptide as to preclude or grossly reduce the efficiency of its binding activity. Necessarily, the interaction of proteins with proteins or with DNA is defined by both conformation and composition of the interactive sites. Changes in composition or length may alter both the local and global conformation of the protein. Consistent with this view, the *ts* domains of the α4 gene, which at the nonpermissive temperature block the transition from α to β and γ genes, have been mapped in many diverse domains of the gene (10, 33). Hence, studies on the functional domains of proteins based on perturbations of their composition must be extended to the entire domain of the gene. Analyses of gene function by truncation are not suitable for this purpose.

ACKNOWLEDGMENTS

These studies were aided by grants from the National Cancer Institute (CA08494 and CA19264), the National Institute for Allergy and Infectious Diseases (AI124009), and the American Cancer Society (MV2W) to the University of Chicago; from the National Institute for Allergy and Infectious Diseases (AI23592) and the National Institute for Dental Research (DE08275) to the University of California; and from The Heart and Lung Institute (P01HL31950) to Scripps Clinic and Research Foundation. J.H.-V. was a predoctoral trainee, National Institute of General Medical Sciences (GM0797).

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