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Inhibition of Herpes Simplex Virus Type 1 DNA Polymerase Activity by Peptides from the UL42 Accessory Protein Is Largely Nonspecific

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To identify regions in the UL42 protein of herpes simplex virus type 1 which affect viral DNA polymerase activity, a series of 96 overlapping pentadecapeptides spanning the entire 488 amino acids of the UL42 protein were synthesized and tested for their ability to inhibit polymerase activity on a primed single-stranded M13 DNA template. Two assays were used: formation of full-length double-stranded M13 molecules and rate of incorporation of deoxyribonucleoside triphosphates. Peptides from five noncontiguous regions of the UL42 protein were found to inhibit herpes simplex virus type 1 polymerase activity in both the presence and absence of UL42 protein. The most active peptides from each region correspond to amino acids 23 to 38 (peptide 6), 64 to 78 (peptide 14), 89 to 102 (peptide 19), 229 to 243 (peptide 47), and 279 to 293 (peptide 57). By two different methods (DNA mobility shift and DNA precipitation), peptides 14, 19, 47, and 57 were found to bind DNA; they most probably inhibit enzyme activity by this mechanism. Peptide 6 did not bind DNA and must act by some mechanism other than competing for DNA. The inhibitory peptides were also tested for activity against mammalian polymerase α and the Klenow fragment of *Escherichia coli* polymerase. Although some limited specificity was demonstrated (up to 10-fold for peptide 6), all the peptides showed significant activity against both polymerase α and *E. coli* polymerase.

The genome of herpes simplex virus type 1 (HSV-1) is a linear double-stranded DNA molecule which contains at least 72 different genes (18) and three origins of viral DNA replication (25, 29). Seven genes (UL5, UL8, UL9, UL29, UL30, UL42, and UL52) are essential for origin-dependent viral DNA synthesis (30). Of these genes, UL30 (18, 19) encodes the catalytic subunit (Pol) of the HSV-1 DNA polymerase (2, 3, 23) and UL42 encodes a double-stranded DNA-binding protein (22) with an apparent M_r of 65,000 (1).

The UL42 gene product is necessary for viral growth and DNA synthesis (14, 16). It physically associates with Pol to form a heterodimer (5, 11, 12) and functions as an accessory protein to increase the rate of incorporation of deoxyribonucleoside triphosphates into activated DNA (10) and to increase Pol processivity on a defined template primer (12, 13). This physical association parallels that previously observed between HSV-2 ICP34,35 and HSV-2 DNA polymerase (27) and is consistent with subsequent evidence demonstrating that the HSV-1 UL42 protein is the serotype equivalent of ICP34,35 (10).

Interest in this virus protein-protein interaction has grown in part because it may be possible to identify compounds which interfere specifically with the interaction and so provide a basis for synthesis of specific antiviral drugs. By immune coprecipitation of the in vitro translation products of the intact UL42 gene and the *pol* (UL30) gene containing various deletions, a fragment of the Pol protein which was both necessary and sufficient for binding of the UL42 protein was identified (8). The fragment encompasses the carboxy-terminal 227 amino acids of the polymerase. More recently, the physical interaction between the two proteins has been shown to be dispensable for catalytic activity of the Pol subunit but essential for increasing its processivity in vitro, for virus replication in vivo (7), and for viral origin-dependent DNA replication in a transient-expression assay (26). These findings strongly support the idea that the interaction between the HSV DNA Pol and UL42 proteins is a valid target of antiviral agents.

The experiments reported here were undertaken to identify regions in the UL42 protein which affect the viral polymerase function. Our approach was to synthesize 96 overlapping pentadecapeptides spanning the entire 488 amino acids of the UL42 protein. These peptides were then tested for their ability to inhibit polymerase activity on a defined template primer in the presence and absence of the UL42 protein. The specificity of inhibitory peptides was investigated by examining their effect on mammalian polymerase α and the Klenow fragment of *Escherichia coli* DNA polymerase I.

MATERIALS AND METHODS

Oligopeptides. Ninety-six pentadecapeptides spanning the entire 488 amino acids of the UL42 protein were synthesized by 9-fluorenylmethoxycarbonyl (Fmoc) chemistry, using a Novabiochem peptide synthesizer. Each peptide overlapped the adjacent one by 10 amino acids, except for those extended or shortened by one amino acid for reasons discussed later. They were all made as peptide amides, except the carboxy-terminal peptide, which was made as an acid. The M_r of each peptide was confirmed by mass spectrometry and their purities were determined by reverse-phase high-pressure liquid chromatography (HPLC) (20). Where indi-

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TABLE 1. UL42 peptides purified by reverse-phase HPLC

Peptide	Sequence	Residues in UL42	% Purity[a]
5	DASLGQPEEGAPCQV	19–33	>99
6	GQPEEGAPCQVVLQGA	23–38	96[b]
7	APCQVVLQGAELNGI	29–43	91[b]
14	RGILIHNTIFGEQVF	64–78	97
15	HNTIFGEQVFLPLEH	69–83	>99
18	SQFSRYRWRGPTAAF	84–98	97
19	YRWRGPTAAFLSLV	89–102	>99
46	REEGVSSSTSTQVQI	224–238	97
47	SSSTSTQVQILSNAL	229–243	95–>99
48	TQVQILSNALTKAGQ	234–248	>99
49	LSNALTKAGQAAANA	239–253	>99
55	VDDCSMRAVLRRLQV	269–283	98
56	MRAVLRRLQVGGGTL	274–288	97
57	RRLQVGGGTLKFFLT	279–293	>99
58	GGGTLKFFLTTPVPS	284–298	95
59	KFFLTTPVPSLCVTA	289–303	94
95	PGAFSAFRGGPQTPY	469–483	>99

[a] Purity was determined by reverse-phase HPLC and checked (peptides 47, 57, and 95 only) by capillary zone electrophoresis, which showed the purified peptide to be homogeneous.

[b] After standing for a few hours, a solution of peptide 6 showed two peaks. The relative proportions varied and could be influenced by time or pH. The nature of the two species was not established, but they may represent different states of oxidation of the cysteine residue. Independently synthesized batches of peptide 6 displayed identical behavior, and peptide 7, which contains the same cysteine residue, showed similar behavior.

cated, peptides were purified by reverse-phase HPLC, using a Dynamax 300A C8 preparative column (internal diameter, 21.4 mm; length, 25 cm; catalog number, 83-323-C) and a gradient of 0 to 95% acetonitrile (Rathburn Chemicals) in water run for 20 min at a flow rate of 10 ml/min. Some peptides were further analyzed by capillary zone electrophoresis (Table 1).

Pol and UL42 proteins. HSV-1 DNA Pol and UL42 proteins were purified exactly as described previously (12) from insect cells infected with recombinant baculovirus. The proteins were stored in aliquots at −70°C until required. DNA polymerase alpha was purified from HeLa Ohio cells by chromatography on Q-Sepharose followed by double-stranded DNA cellulose. The Klenow fragment of *E. coli* polymerase I was produced from a plasmid construct (a gift of C. Joyce and N. Grindley) as described previously (15).

DNA polymerase assays. (i) HSV-1 DNA polymerase. HSV-1 DNA polymerase activity was assayed by using a singly primed, single-stranded circular M13 DNA template under conditions described previously (12) with minor modifications. The template was prepared by hybridizing 1 pmol of synthetic oligonucleotide primer (New England BioLabs sequencing primer no. 1211: 5′-GTAAAACGACGGCCA GT) to 400 fmol of M13mp18 DNA (New England BioLabs) in 20 μl of 20 mM Tris-HCl (pH 7.5)–25 mM NaCl. The mixture was incubated at 65°C for 2 min and allowed to cool slowly to room temperature for 2 h.

The assay was performed at 37°C by using a reaction volume of 50 μl containing 20 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES) (pH 7.6), 1 mM $MgCl_2$, 4% glycerol, 0.1 mM EDTA, 1 mM dithiothreitol, 75 mM NaCl, 0.5 mM ATP, three unlabeled deoxyribonucleoside triphosphates at 60 μM and the fourth α-^{32}P-labeled nucleotide (either dATP or dCTP at 9×10^4 cpm/pmol) at 2 μM, 2.5 μg of bovine serum albumin (BSA), and Pol and UL42 proteins as described below. Primed M13 DNA was used at 4 nM (10 μg/ml) for measuring the activity of Pol alone and at 0.4 nM for all other assays. Peptides were included as indicated, and the mixture was preincubated at 37°C for 10 min (3 min in assays of full-length M13 formation). Reactions were initiated by the addition of α-^{32}P-labeled deoxynucleoside triphosphate.

To measure the synthesis of full-length M13 DNA, the reactions were terminated after 30 min by the addition of 150 μl of stop solution: 3 μg of sonicated calf thymus DNA in 0.67% sodium dodecyl sulfate–300 mM NaCl. The reaction products were extracted with an equal volume of phenol followed by chloroform-isoamyl alcohol (24:1), precipitated in ethanol, redissolved in 40 μl of alkaline loading buffer (100 mM NaOH, 1 mM EDTA, 2.5% Ficoll, bromophenol blue to color), and analyzed by gel electrophoresis in alkaline 1% agarose gels. The DNA was denatured to single strands under these conditions. The radioactivity in the gel was visualized by autoradiography. The amount of full-length product was quantitated by densitometry, using a Hoefer GS-300 scanning densitometer and GS-360 data analysis system. Double-stranded M13 DNA linearized with *Sma*I and end labeled with ^{32}P was used as a size marker on all gels.

To measure the rate of DNA synthesis, 10-μl samples were removed at intervals and spotted onto Whatman DE81 ion-exchange filters that had been previously soaked in 0.1 M EDTA and air dried. The discs were given three 10-min washes with 5% (wt/vol) Na_2HPO_4, two 5-min washes with water, and finally two 30-s washes with industrial methylated spirits. They were dried and counted in a Beckman LS5000CE scintillation counter.

(ii) Mammalian DNA polymerase α. Mammalian DNA polymerase α activity was assayed at 37°C by using 200 μl of 75 mM Tris-HCl buffer (pH 7.5) containing 6.5 mM $MgCl_2$; 83 μM each dATP, dCTP, and dGTP; 1.67 mM 2-mercaptoethanol; 11 μg of activated calf thymus DNA per ml; 0.42 mg of BSA per ml; 3.4 μM [^{3}H]dTTP (10.2 Ci/mmol); and 30 mM NaCl carried over into the assay from the enzyme solution. Peptides were included as appropriate, and assay mixtures were preincubated at 37°C for 10 min in the absence of [^{3}H]dTTP prior to initiation of reaction with the labeled nucleotide. Samples of 20 μl were removed at intervals over the first 16 min of reaction and spotted onto a DEAE-filter mat previously soaked in 0.1 M Na_4EDTA and air dried.

(This pretreatment of the filter had the effect of immediately stopping the polymerase reaction and prevented high blank counts.) The filter mat was washed as described above and counted in an LKB beta-plate scintillation counter.

(iii) Klenow fragment of *E. coli* polymerase I. The activity of the Klenow fragment of *E. coli* polymerase I was assayed at 37°C by using a reaction volume of 50 μl containing 50 mM Tris-HCl (pH 7.5); 10 mM $MgCl_2$; 1 mM dithiothreitol; 60 μM each dGTP, dATP, and dTTP; 20 μM [α-^{32}P]dCTP (9×10^3 cpm/pmol); 20 μg of BSA; 4 nM primed M13 DNA; and 0.1 U of enzyme. Peptides were included as indicated, and the mixture was preincubated at 37°C for 10 min. Reactions were initiated by the addition of [α-^{32}P]dCTP. Samples were removed at intervals onto Whatman DE81 filters as described above.

DNA mobility shift assay. A duplex oligonucleotide with the sequence

5′-GATCCGCGAAGCGTTCGCACTTCGTCCCA
GCGCTTCGCAAGCGTGAAGCAGGGTCTAG-5′

was purified, ^{32}P end labeled, and blunt ended as described previously (28). The assay was performed at 37°C by using a reaction volume of 20 μl containing 1 ng of radiolabeled DNA. The same buffer conditions were used as for the HSV-1 polymerase assay: 20 mM HEPES (pH 7.6), 1 mM $MgCl_2$, 4% glycerol, 0.1 mM EDTA, and 1 mM dithiothreitol. Salt and peptides were added as described below. After 20 min, 5 μl of loading buffer (25% glycerol, 10 mM dithiothreitol, 0.01% bromophenol blue in TBE [90 mM Tris-borate, 1 mM EDTA]) was added, and the samples were analyzed by electrophoresis in 8% polyacrylamide gels (acrylamide/*N*,*N*′-methylene bisacrylamide ratio, 40:1) containing TBE. Gels were run in TBE at 120 V for 3 h and dried, and the radioactivity was visualized by autoradiography.

DNA precipitation assay. Primed M13 DNA and activated salmon sperm DNA were labeled with ^{32}P by incubating 2 μg of DNA in a volume of 180 μl for 30 min with Klenow polymerase under the conditions described above for assaying Klenow polymerase. The assay was performed by using a volume of 50 μl containing 20 ng of radiolabeled DNA. Peptides were added as described below, and the mixture was incubated for 20 min at 37°C. The tubes were spun for 10 min at 13,000 × *g* in a bench centrifuge, and then a 10-μl sample of the supernatant was removed onto Whatman DE81 filters as described above and the radioactivity was measured.

RESULTS

Oligopeptides. The peptides used were numbered from 1 to 96: the *n*th peptide comprised amino acids $5n - 6$ to $5n + 8$ in the published amino acid sequence of UL42 (19). There were a few exceptions: peptide 1 contained residues 1 to 13; peptides 6, 22, 38, and 67 were 16 amino acids long and contained one additional residue at the amino terminus to avoid peptides with an amino-terminal glutamine, which could result in pyroglutamate formation; and peptides 3, 19, 35, and 64 were correspondingly shortened to 14 amino acids. A few peptides (peptides 12, 13, 43, 54, and 61) were poorly soluble and could not be adequately studied.

Peptide inhibition of synthesis of full-length M13 DNA molecules by Pol/UL42. The concentration of UL42 and template in the assay and the reaction time were chosen so that any inhibition of processive activity would be detected by a reduction in the amount of full-length DNA product. First, as observed previously (12), the amount of full-length product was proportional to the UL42 concentration up to an amount above which there was little increase in product: this concentration was used in further experiments. Second, the reaction proceeded such that after an initial 7-min lag, which was due to the time taken by the polymerase to traverse the template, the rate of formation of full-length product was linear for 40 min: a time of 30 min was chosen. Third, the concentration of template used in the reactions (0.4 nM) was five times that below which the template became rate limiting.

Peptides were initially screened at a concentration of 50 μM, without purification, for their effect on the synthesis of full-length M13 DNA. Reactions with Pol/UL42 and Pol alone were always included as controls. Figure 1 shows the results of two such experiments, which identify peptides 47, 48, 57, 58, and possibly 46 as inhibitory. By using this assay, peptides 6, 7, 8, 14, 15, 18, 19, 47, 48, 57, and 58, identifying five discrete regions, were found to be inhibitory. Those which appeared most inhibitory were purified for further study. Figure 2 shows data for peptides 6, 14, 47, and 48 from which 50% inhibitory concentrations (IC_{50}s) of 3.5, 7.5, 0.8, and 9 μM, respectively, were determined. The values for peptides 6, 14, 19, 47, 57, and 95 are shown in Table 2.

Peptide inhibition of synthesis of full-length M13 DNA by Pol alone. In the absence of UL42 protein, the DNA Pol subunit is processive, although to produce the same amount of full-length product nearly 10-fold greater amounts of Pol alone than of Pol/UL42 are required (12). We therefore tested peptides for inhibition of full-length product formation by Pol alone. Again, we chose the assay conditions so that any inhibition would be reflected in a reduction in the amount of full-length product. In particular, the formation of full-length product (i) was linear with time up to 35 min after an initial lag and (ii) increased with Pol concentration. A reaction time of 30 min and a Pol concentration five times that used to assay Pol/UL42 were chosen.

The results with purified peptides 5, 47, 56, 57, and 95 at 50 μM are presented in Fig. 3a and show peptides 47 and 57 to be completely inhibitory at this concentration. An IC_{50} of 1.5 μM for inhibition by peptide 47 was obtained by densitometric analysis of the data shown in Fig. 3b. This value and those for other peptides are listed in Table 2. Two shorter M13 DNA products (5.0 and 3.0 kb) accumulated to a greater extent in the absence of UL42 protein and probably arose from the Pol "pausing" at specific sites on the template (compare Fig. 1 and 3 and results in reference 12). Densitometric analysis showed that these species and the full-length product are inhibited to the same extent (data not shown). Synthesis of these shorter molecules by Pol/UL42 was also inhibited by the peptides (Fig. 1), as was the accumulation of M13 DNA products of 0.5 to 0.7 kb.

Peptide inhibition of the rate of DNA synthesis by Pol and Pol/UL42. The action of the above-mentioned peptides was further investigated by determining their effect on the rate of incorporation of [α-^{32}P]dCTP into DNA. To achieve linear incorporation, the assay was modified as follows: (i) peptides were preincubated for 10 min at 37°C with the assay mixture lacking dCTP; and (ii) for Pol alone, the M13 template concentration was increased to ensure that it was not limiting. The K_m values for Pol for the M13 template in the presence and absence of UL42 were determined and found to be 0.1 and 1 nM, respectively (data not shown). These values are consistent with a 5- to 10-fold increase in the affinity of Pol for a primed template in the presence of UL42 (11a). Accordingly, M13 template was used at 0.4 nM for

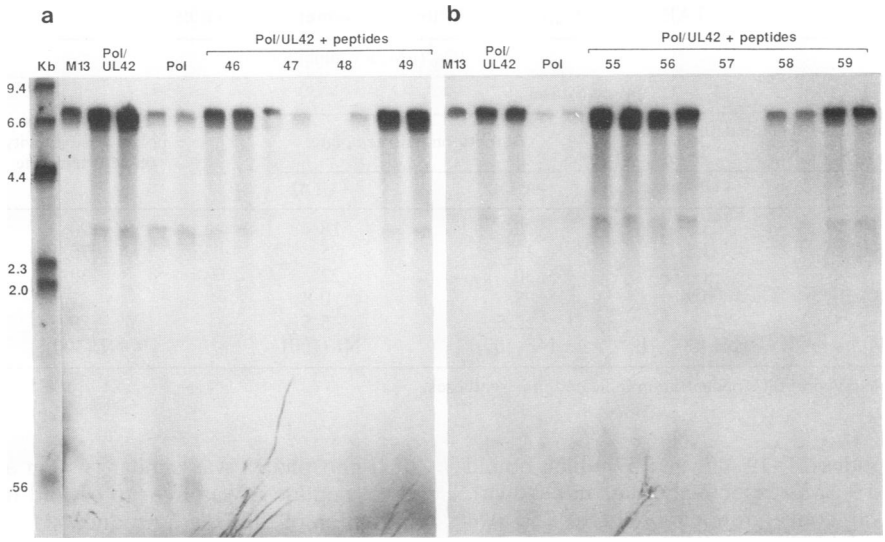

FIG. 1. Effect of peptides (50 μM) on the formation of full-length M13 by HSV-1 Pol/UL42. Two separate experiments (a and b) are shown; in both of these, the formation of full-length M13 in the presence (Pol/UL42) and absence (Pol) of UL42 protein is demonstrated. The effect of peptides 46, 47, 48, and 49 on the formation of full-length M13 by Pol/UL42 (Pol/UL42 + peptides) is shown in panel a, and the effect of peptides 55, 56, 57, 58, and 59 is shown in panel b. All reactions were performed in duplicate. Also shown are molecular size markers: λ DNA digested with *Hin*dIII (Kb; part a) and full-length, linearized M13 DNA (M13; parts a and b), both end labeled with ^{32}P. The products of the reaction and marker DNAs were analyzed by electrophoresis in alkaline 1% agarose gels.

Pol/UL42 and at 4 nM for Pol alone, which results in approximately equal rates of incorporation by Pol/UL42 and Pol alone.

The inhibition of polymerase activity by peptides in the presence and absence of UL42 was determined. The progress plots at various concentrations of all tested peptides were linear over the first 10 minutes of reaction (data not shown). These rates were plotted (Fig. 4) to determine IC_{50}s (Table 2).

Peptide inhibition of *E. coli* polymerase I and mammalian polymerase α activities. The specificity of the peptides for HSV-1 polymerase was assessed by testing their effect on mammalian polymerase α and on the Klenow fragment of *E. coli* polymerase I, whose catalytic domain bears no sequence homology to HSV Pol (24).

Incorporation of [α-^{32}P]dCTP into DNA by the *E. coli* enzyme was linear under the conditions described. The K_m for the Klenow polymerase for the M13 template was 1.6 nM (data not shown), and a concentration of 4 nM was used. Progress plots for the Klenow polymerase in the absence of peptides and in the presence of peptides 6, 19, 57, and 95 were linear over the first 10 min of the reaction. Inhibition by peptides 14 and 47 increased with time, particularly at the higher peptide concentrations (data not shown). To determine the IC_{50}s of these two peptides, we used the rates of incorporation between 2.5 and 5 min; the derived IC_{50}s are given in Table 2. Incorporation of [^{3}H]dTTP into activated calf thymus DNA by mammalian polymerase α in the absence of peptides and in the presence of different concentrations of peptides 6, 14, 19, 47, and 57 was linear with respect to time. The derived IC_{50}s are shown in Table 2.

Interaction of peptides with DNA. One possible mechanism by which the inhibitory peptides could interfere with polymerase activity would be by blocking its binding to DNA. A gel mobility shift assay was used to investigate their DNA-binding properties. Peptides were tested at a concentration of 50 μM, under the same buffer conditions as used for the HSV-1 DNA polymerase assays, for their ability to form complexes with a synthetic duplex oligodeoxynucleotide. Peptides 14, 18, 19, 47, 48, and 57 formed complexes which had various mobilities, whereas peptides 6, 7, 15 and 95 did not bind (Fig. 5). Of the other peptides shown in Table 1, peptides 46, 55, 56, 58, and 59 formed complexes whereas peptides 5 and 49 did not. Identical binding was seen in the presence of 76 mM NaCl and in the absence of salt (data not shown). Further evidence for the interaction of peptides 14, 19, 47, and 57 with DNA was provided by the observation that they precipitated both M13 DNA and activated salmon sperm DNA (Table 3). Also, in agreement with the results of the mobility shift assays, peptide 6 did not precipitate these DNAs.

The minimum amount of peptide required to produce a detectable shift in the mobility of the DNA was determined by using serial threefold dilutions of the peptide. The lowest

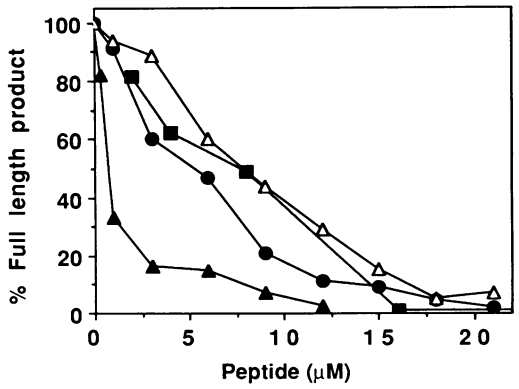

FIG. 2. Inhibition of full-length product formation by peptides 6 (●), 14 (■), 47 (▲), and 48 (△).

TABLE 2. IC_{50}s for inhibition of polymerase activities

Peptide	IC_{50} (μM) for inhibition of:					
	HSV polymerase				*E. coli* Klenow polymerase activity on M13 template	Mammalian polymerase α activity
	Formation of full-length M13 DNA		Activity on M13 template			
	−UL42	+UL42	−UL42	+UL42		
6	3.5	3.5	20	18	190	43
14	7.5	7.5	13	5	75	21
19	20	9	30	22	32	19
47	1.5	0.8	3	0.8	21	3.6
57	9	12	12.5	5.5	29	70
95	NI (50)[a]	NI (50)	NI (100)	NI (100)	NI (300)	NI (200)

[a] NI, no inhibition was observed at the concentration indicated in parentheses.

concentrations of peptides 14, 19, 47, and 57 which bound DNA were 3, 9, 1, and 9 μM, respectively (data not shown). However, peptide 6 at concentrations of up to 250 μM showed no evidence of binding to DNA and is therefore acting by another mechanism.

DISCUSSION

The studies reported here have identified peptides from five separate regions of the UL42 protein which interfere with HSV polymerase activity. The inhibitory peptides blocked both formation of full-length M13 DNA and incorporation of triphosphates into a primed M13 DNA template. The most potent was peptide 47, and the least potent was peptide 19. More reliance should be attached to the values obtained from the incorporation experiments for the following reasons. First, the values for the formation of full-length product are based on a single datum point (taken at 30 min). Second, it is not known whether the rate of formation of product in the presence of peptide is linear. Third, densitometry is less accurate than is radioactivity counting in a scintillation counter. In general, the level of inhibition by the peptides of formation of full-length product or incorporation of triphosphates was similar (within a factor of 2.4). However, peptide 6 was five- to sixfold more inhibitory of the formation of full-length product.

The experiments reported here were stimulated by the observation that HSV ribonucleotide reductase is specifically inhibited by the nonapeptide YAGAVVNDL, corresponding to the carboxy terminus of the small subunit of the enzyme (4, 9). We were interested in whether this phenomenon was peculiar to ribonucleotide reductase or whether similar inhibition of other interacting subunits could be obtained by using peptides corresponding to one of the subunits. The question is of interest because the YAGAV VNDL peptide acts by competing for a site on the large subunit to which the small subunit binds and so inhibits the normal association of the two subunits (4, 6, 9, 17, 21); in principle, any biological process which depends on the interaction between two subunits could be blocked by a compound which interferes with that interaction.

The data presented here provide no evidence that any of the inhibitory peptides act by disruption of the Pol/UL42 interaction, although the possibility that peptide 6 does so has not been excluded. A peptide which acted in this manner

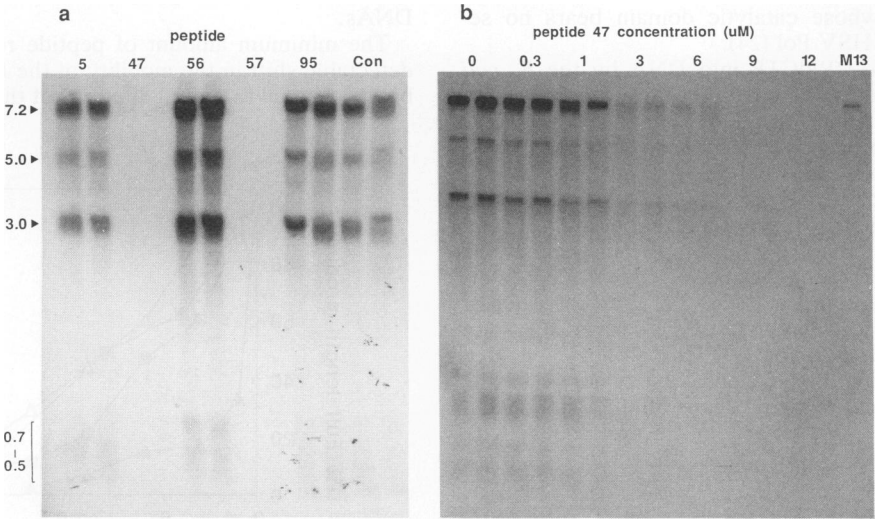

FIG. 3. Effect of peptides on formation of full-length M13 by DNA Pol alone. (a) Effect of peptides 5, 47, 56, 57, and 95, all at 50 μM, as well as a control lacking peptide (Con). (b) Products formed in the presence of various concentrations of peptide 47. All reactions were performed in duplicate. The single track on the right shows full-length, linearized M13 DNA end labeled with ^{32}P. The DNAs were electrophoresed in alkaline 1% agarose gels. The numbers on the left of the figure give the sizes of the products in kilobases.

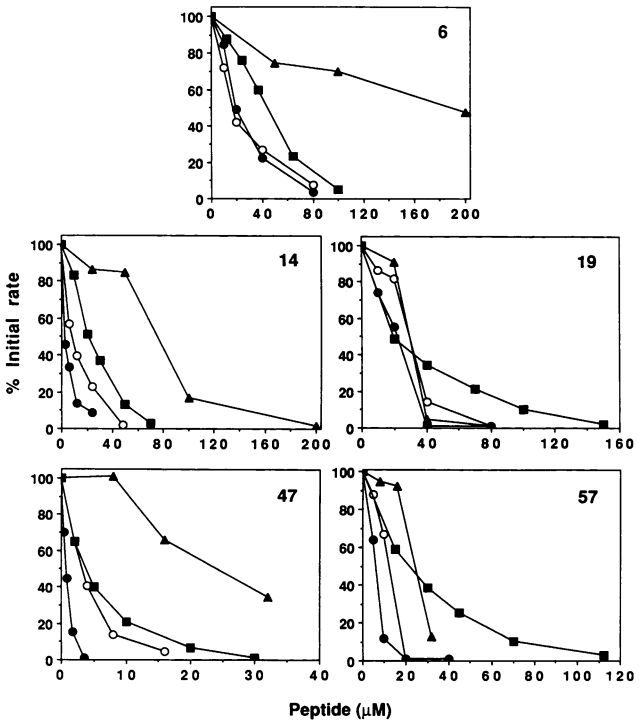

FIG. 4. Comparison of the inhibition of HSV-1 Pol (○), Pol/UL42 (●), mammalian polymerase α (■) and Klenow polymerase (▲) by peptides 6, 14, 19, 47, and 57. The rate of incorporation of [α-^{32}P]dCTP into M13 DNA in the presence of peptide is expressed as a percentage of the uninhibited rate.

might have been expected to reduce the level of Pol/UL42 activity to that of Pol alone and to have no effect on Pol itself. Indeed, there is clear evidence that peptides 14, 19, 47, and 57 act by interacting with the primed M13 DNA template. The minimum concentrations of these peptides required to bind DNA (3, 9, 1, and 9 μM, respectively) are similar to the IC_{50}s for inhibition of HSV polymerase (Table 2), suggesting that their DNA-binding activity accounts for the observed inhibition. Moreover, the observation that the

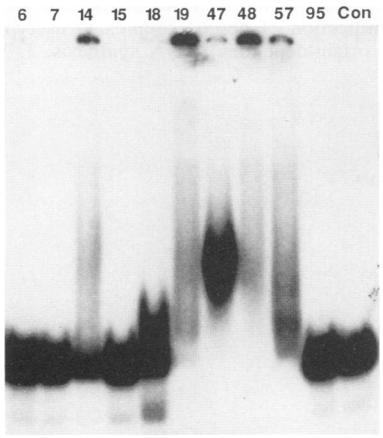

FIG. 5. Mobility of a duplex oligonucleotide in the absence of peptide (Con) and in the presence of peptides 6, 7, 14, 15, 18, 19, 47, 48, 57, and 95, all at 50 μM.

TABLE 3. Precipitation of DNA by peptides

Peptide	% of DNA remaining in solution:	
	M13 DNA	Activated salmon sperm DNA
None	100	100
6	109.7	132.7
14	0.2	1.0
19	0.02	0.6
47	2.5	2.7
57	0.1	0.1
95	53.5	30.9

rate of DNA synthesis by Pol/UL42 is more inhibited by peptides 14, 19, 47, and 57 than is the rate of synthesis by Pol alone (Fig. 4) is probably a reflection of the lower concentration of DNA used in the Pol/UL42 assay (0.4 nM) than that used in the Pol assay (4 nM).

Peptides 6, 14, 47, and 57 did display some limited specificity for inhibition of the different polymerases tested (Fig. 4; Table 2). For peptides 14, 47, and 57, the specificity possibly reflects the different affinities of the enzymes for DNA. It is noteworthy that peptide 6, which does not bind to DNA, shows the same activity against Pol/UL42 and Pol alone. For peptide 6, which inhibits by some means other than binding to DNA, the specificity must be due to another mechanism. Of all the peptides, peptide 6 shows the greatest specificity for the HSV polymerase, being 10-fold less active against Klenow polymerase and 2-fold less active against mammalian polymerase α. Whether this reflects a specificity for α-like polymerases remains to be tested.

The limited specificity of all five peptides against the mammalian enzyme renders them unsuitable as lead compounds for antiviral drug development. Our results suggest that other approaches such as saturation mutagenesis of UL42, to find the regions of the protein involved in its interaction with polymerase, will also not lead to the identification of specific inhibitory UL42 peptides with IC_{50}s of less than 20 μM. It is possible that the insoluble peptides 12, 13, 43, 54, and 61 contain sequences which demonstrate specific inhibition. However, we consider this possibility unlikely because all the sequences present in the insoluble peptides have been tested in adjacent soluble ones since there is an overlap of 10 amino acids across the peptides. These considerations do not exclude the possibility of finding other molecules which specifically inhibit the interaction between the Pol and UL42 proteins.

Whether the finding that peptides 14, 19, 47, and 57 bind DNA indicates that they correspond to regions of the UL42 protein involved in binding DNA is currently under investigation, as is the different mechanism of action of peptide 6.

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