Secondary Structure and Structure-Activity Relationships of Peptides Corresponding to the Subunit Interface of Herpes Simplex Virus DNA Polymerase*

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The interaction of the catalytic subunit of herpes simplex virus DNA polymerase with the processivity subunit, UL42, is essential for viral replication and is thus a potential target for antiviral drug discovery. We have previously reported that a peptide analogous to the C-terminal 36 residues of the catalytic subunit, which are necessary and sufficient for its interaction with UL42, forms a monomeric structure with partial α-helical character. This peptide and one analogous to the C-terminal 18 residues specifically inhibit UL42-dependent long chain DNA synthesis. Using multidimensional 1H nuclear magnetic resonance spectroscopy, we have found that the 36-residue peptide contains partially ordered N- and C-terminal α-helices separated by a less ordered region. A series of “alanine scan” peptides derived from the C-terminal 18 residues of the catalytic subunit were tested for their ability to inhibit long-chain DNA synthesis and by circular dichroism for secondary structure. The results identify structural aspects and specific side chains that appear to be crucial for interacting with UL42. These findings may aid in the rational design of new drugs for the treatment of herpesvirus infections.

Specific protein-protein interactions play a crucial role in virtually all cellular processes and are often mediated by discrete peptide motifs (1). Peptide mimetics capable of selectively disrupting these interactions may have significant therapeutic potential in a wide variety of diseases. A promising target for this type of drug is the DNA polymerase encoded by herpes simplex virus, which is a heterodimer consisting of the catalytic subunit (Pol)1 and the processivity factor UL42. Although Pol alone exhibits catalytic activity, both subunits are required for viral replication (2, 3). In addition, UL42 mutants specifically impaired for Pol binding are unable to mediate long-chain DNA synthesis or to complement viral replication (4). Pol mutants specifically impaired for UL42 binding are also defective for long-chain DNA synthesis and viral replication (5, 6). Because the interaction between Pol and UL42 is specific and essential, it is an attractive target for the development of new anti-HSV drugs.

Functional mapping of Pol has indicated that a segment containing the C-terminal 32–40 amino acid residues is required for UL42 binding (5–8). This region of Pol is not highly conserved among any other herpesvirus or cellular DNA polymerase and is not necessary for catalytic activity. Previous studies demonstrated that a peptide corresponding to the C-terminal 36 amino acid residues of Pol, designated peptide A (Pol residues 1200–1235, Fig. 1), could selectively inhibit the ability of UL42 to stimulate long-chain DNA synthesis (9). Peptide A2 and a fusion peptide corresponding to the C-terminal 27 residues of Pol (10) bind specifically to UL42, indicating that this small region of Pol is both necessary and sufficient for UL42 binding. A shorter peptide corresponding to the last 18 residues of Pol, designated peptide E (Pol residues 1218–1235, Fig. 1), also binds to UL42 and is a specific inhibitor of UL42-mediated DNA synthesis (9). Peptide E is only 4–10-fold less potent an inhibitor than peptide A, suggesting that the C-terminal 18 residues of Pol are most important for UL42 binding. Because the region of Pol responsible for interacting with UL42 appears to be small and discrete, it may be a good target for drug discovery.

Circular dichroism studies indicated that peptides A and E are partially helical and suggested that peptide A might fold into a helix-loop-helix structure4 with the second helix contained within peptide E (9). In order to better understand the nature of the Pol/UL42 interaction, we have further studied the structure of peptide A by multidimensional 1H NMR and CD spectroscopy and, using mutant peptides, have identified discrete elements of this structure that are important for inhibition of Pol/UL42 function. This information may aid in the inducible domain: CBP, CREB-binding protein; KIX, pKID-binding domain of CBP; HMQC, heteronuclear multiple quantum coherence; pKID, phosphopeptide motif of CREB that binds to the KIX domain of CBP.

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1 The abbreviations used are: Pol, polymerase; HSV, herpes simplex virus; IQC-COSTY, double quantum-filtered COSY; NOE, nuclear Overhauser enhancement; NOESY, NOE spectroscopy; TOCSY, total correlation spectroscopy; TOCSY-NOESY, homonuclear three-dimensional NMR experiment combining successive TOCY and NOESY mixing schemes; CREB, cAMP-response element-binding protein; KID, kinase-inducible domain: CBP, CREB-binding protein; KIX, pKID-binding domain of CBP; HMQC, heteronuclear multiple quantum coherence; pKID, phosphopeptide motif of CREB that binds to the KIX domain of CBP.

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The use of “helix-loop-helix” is meant as a description of the peptide structure and is not meant to suggest any structural or evolutionary relationship to the HLH class of DNA binding proteins.
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EXPERIMENTAL PROCEDURES

Materials—Peptide A was synthesized as described previously (9). Alanine scan mutants of peptide E were prepared via Fmoc (N-(9-fluorenlyl)methoxy carbonyl) solid phase peptide synthesis on an Advance Chem Tech 396 MPS Synthesizer (Louisville, KY) at 50 μm scale employing the manufacturer’s standard coupling procedure (DIPCDI/HOBt or HBTU/HOBt/DIEA). Cleavage and deprotection of peptideyl resins were performed with a cleavage mixture composed of trifluoroacetic acid/anisole/thioanisole/ethanedithiol/water (94:2:2:1:1) at room temperature for 2.5 h. Crude peptide was separated from resin by filtration, precipitated with methyl t-butyl ether, collected by centrifugation, and dried under reduced pressure. Purification was performed on a high pressure liquid chromatography reverse-phase C18 column (Rainin Dynamax, 10 × 250 mm) using an acetonitrile gradient in 0.1% trifluoroacetic acid (v/v). HSV Pol and UL42 were purified from insect cells infected with the appropriate recombinant baculoviruses as described previously (11, 12). Poly(dA) template and oligo(dT) primer were purchased from Amersham Pharmacia Biotech. TTP was purchased from Roche Molecular Biochemicals, and [32P]TTP was obtained from NEN Life Science Products.

CD Spectroscopy—Lyophilized peptides were resuspended in 10 mM potassium fluoride and adjusted to pH 8 with KOH. Spectra were recorded at the indicated peptide concentrations with an Aviv 62DS SpectroPolarimeter at 0 °C in a 0.1-cm path length cuvette. Wavelength scans were recorded at 1-nm intervals with a 5-s averaging time, and 5–10 scans were averaged. Peptide concentrations were determined by quantitative amino acid analysis. Unfolding curves were obtained by monitoring mean residue ellipticity at 222 nm as a function of the concentration of guanidine-HCl and temperature. These experiments employed an automated titrator as described by the vendor.

NMR Spectroscopy—NMR spectra were obtained in aqueous solution of 50 mM KCl at pH 5.6 as adjusted with HCl. Spectra were obtained at 500 and 600 MHz using Varian Unityplus spectrometers. One-dimensional spectra were obtained at successive temperatures (4–40 °C) and peptide concentrations (0.2–4.6 mM). Chemical shifts were found to be independent of peptide concentration at the concentrations tested; resonance line widths were consistent with a monomeric fragment. Two-dimensional NOESY (mixing times 200 and 400 ms), TOCSY (mixing time 55 ms), DQF-COSY, and 13C-1H HMQC spectra were obtained in each case. Three-dimensional 1H-1H TOCSY-NOESY spectra were obtained with respective mixing times of 55 and 400 ms. Three-dimensional spectra were processed using Varian software with linear prediction. For the 1H-1H TOCSY-NOESY spectra, the observed three-dimensional data matrix was 64 × 128 × 1024 and extended by linear prediction to 256 × 512 × 1024. Use of this data in sequential assignment was performed as described (13). Random-coil values of 1H NMR chemical shifts were obtained by Wuthrich (14). 1H 1HN, coupling constants were extracted from DQF-COSY spectra in H2O as described previously (15).

Analytical Ultracentrifugation—Equilibrium sedimentation analysis of peptide E was performed with a Beckman XL-A analytical ultracentrifuge using double sector cells with charcoal-filled Epon centerpieces and sapphire windows. The peptide was dissolved in 100 mM sodium phosphate at pH 8. The analysis was performed at 20 °C at 60,000 rpm. The distribution of peptide at various radii was assayed by absorbance at 230 nm, with a scan taken every 4 h until equilibrium was attained. Equilibrium data were analyzed using nonlinear least squares methods using a modified version of Igor Pro (Wavemetrics, Lake Oswego, OR) running on a Macintosh computer as described (16). The partial specific volume of peptide E was calculated from the amino acid composition.

Polymerase Assays—Reaction mixtures contained 50 mM Tris-Cl (pH 7.6), 100 mM (NH4)2SO4, 3 mM MgCl2, 0.1 mM EDTA, 1 mM dithiothreitol, 0.1 μg of bovine serum albumin, 4% glycerol, 0.25 μg of poly dA-dT primer-template, 50 μM [32P]TTP (5 Ci/mmol), 100 fmol of HSV Pol, 200 fmol of UL42, and varying concentrations of peptide inhibitors in a final volume of 25 μl. Reactions were carried out at 37 °C for 5–10 min. Reactions were stopped by placing them on ice and adding 5 μl of alkaline loading buffer (2 mM EDTA, 50 mM NaOH, 2.5% glycerol, 0.025% brom cresol green) and were then loaded onto a 4% alkaline agarose gel. Gels were dried overnight and used to expose film and phosphorescence screens. Because Pol alone added only one or two nucleotides to the 15-base primer, newly synthesized DNA larger than 18 bases was defined as long-chain and quantified using a Molecular Dynamics PhosphorImager.

RESULTS

Our results are presented in two parts. A nascent helix-loop-helix structure in peptide A is first characterized at low temperature by circular dichroism and multidimensional 1H NMR spectroscopy. Structure-activity relationships in the isolated C-terminal segment are then investigated using alanine scan variants of this region. Peptide A Contains Ordered Structure at Low Temperature Whose Unfolding Is Not Cooperative—As demonstrated previously (9) and shown in Fig. 2A, the CD spectrum of peptide A exhibits a partial α-helical signature with a helix content of 20–30% inferred from mean residue ellipticity at 222 nm. Based on analytical ultracentrifugation studies and the lack of concentration dependence for helicity (9), peptide A is monomeric in solution. To investigate the strength of potential intramolecular interactions, peptide unfolding was monitored at 222 nm as a function of temperature (Fig. 2B) and denaturant (guanidine-HCl) concentration at 4 °C (Fig. 2C). The temperature dependence of mean residue ellipticity at 222 nm exhibited an incremental and nonsigmoidal (noncooperative) attenuation of helix content with increasing temperature, and curves were

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FIG. 1. Amino acid sequence of peptides A and E.

FIG. 2. A, native CD spectrum of peptide A. Denaturation titration of peptide A with temperature (B) and with increasing concentrations of guanidine-HCl (C) is shown. Peptide concentrations were 5 μM in A and 50 μM in B in 50 mM KCl at pH 5.5.

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identical at two peptide concentrations (30 and 70 μM). Unfolding occurred without detectable cooperativity. Similarly, the guanidine-HCl unfolding was consistent with a monomeric unfolding transition and a lack of cooperative higher order structure.

Peptide A Forms a Nascent Helix-Loop-Helix Motif at Low Temperature—1H NMR studies were undertaken to delimit regions of ordered structure within peptide A. One-dimensional NMR spectra were independent of peptide concentration in the range 0.2–4.6 mM and exhibited sharp peaks and a range of shifts characteristic of an ordered or partially ordered structure (Table I). The “fingerprint” regions of the two-dimensional NOESY spectrum (Fig. 3A) and TOCSY and DQF-COSY spectra enabled complete sequential assignments; ambiguities were resolved by use of three-dimensional TOCSY-NOESY spectroscopy (15). Two discrete helices (residues 2–6 and 18–28) could be defined by strings of successive (i, i+3) d_{NN} NOEs in this region. In addition, the C-terminal helix appeared to extend in a less regular fashion through residue 36. Residues 7–17 were not well ordered. The pattern of 3J_{HNα} coupling constants (Table II) reflects the partial ordering of the peptide; even in α-helical segments, couplings were greater than 5 Hz, presumably as a result of motional averaging. The helical segments are likely to be stabilized in each case by at least one (i,

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**Table I**

Chemical shifts of assigned 1H NMR resonances (at pH 5.4 and 4 °C)

| Position | Residue | NH       | C_H | CαH     | Others        |
|----------|---------|----------|-----|---------|---------------|
| 1        | Asp     | 8.50     | 4.55| 2.69, 2.61| C_H 1.02, 0.99|
| 2        | Asp     | 8.63     | 4.60| 2.77, 2.68|               |
| 3        | Val     | 8.08     | 3.89| 2.15     |               |
| 4        | Ala     | 8.34     | 4.15| C_H 1.45 |               |
| 5        | Ala     | 8.21     | 4.20| C_H 1.45 |               |
| 6        | Arg     | 8.12     | 4.10| 1.91, 1.75| C_H 1.60, C_H 3.22, 3.2 N_H 7.50 |
| 7        | Leu     | 8.05     | 4.22| 1.59, 1.51| C_H 1.73, C_H 0.90, 0.85 |
| 8        | Arg     | 8.21     | 4.20| 1.80, 1.72| C_H 1.62, C_H 3.17, 3.17 N_H 7.36 |
| 9        | Ala     | 8.08     | 4.16| C_H 1.46 |               |
| 10       | Ala     | 8.13     | 4.21| C_H 1.45 |               |
| 11       | Gly     | 8.18     | 3.93, 3.87| 3.17, 3.03| C2,6H 7.27, C3,5H 7.35 |
| 12       | Phe     | 8.18     | 4.59|          |               |
| 13       | Gly     | 8.50     | 3.87, 3.87|          |               |
| 14       | Ala     | 8.18     | 4.34| C_H 1.38 |               |
| 15       | Val     | 8.33     | 4.10| 2.09     | C_H 0.98, 0.98 |
| 16       | Gly     | 8.64     | 3.96, 3.96|            |               |
| 17       | Ala     | 8.40     | 4.30| C_H 1.41 |               |
| 18       | Gly     | 8.59     | 3.95, 3.95|            |               |
| 19       | Ala     | 8.26     | 4.41| C_H 1.40 |               |
| 20       | Thr     | 8.40     | 4.35| 4.49     | C_H 1.31      |
| 21       | Ala     | 8.76     | 4.29| C_H 1.47 |               |
| 22       | Glu     | 8.58     | 4.12| 2.96, 2.00| C_H 2.34, 2.34 |
| 23       | Glu     | 8.20     | 4.10| 2.18, 2.08| C_H 2.35, 2.35 |
| 24       | Thr     | 8.34     | 4.02| 4.26     | C_H 1.24      |
| 25       | Arg     | 8.05     | 4.16| 1.93, 1.78| C_H 1.63, C_H 3.22, 3.22 N_H 7.58 |
| 26       | Arg     | 8.21     | 4.20| 1.87, 1.72| C_H 1.69, C_H 3.17, 3.17 N_H 7.36 |
| 27       | Met     | 8.11     | 4.30| 2.18, 2.18| C_H 2.69, 3.57 C_H 2.08 |
| 28       | Leu     | 8.05     | 4.22| 1.80, 1.59| C_H 1.73 C_H 0.91, 0.86 |
| 29       | His     | 8.27     | 4.64| 3.34, 3.22| C_H 8.59, C_H 7.32 |
| 30       | Arg     | 8.28     | 4.27| 1.82, 1.75| C_H 1.64, C_H 3.22, 3.22 N_H 7.22 |
| 31       | Ala     | 8.42     | 4.21| C_H 1.32 |               |
| 32       | Phe     | 8.20     | 4.59| 3.17, 3.03| C2,6H 7.27, C3,5H 7.34 |
| 33       | Asp     | 8.32     | 4.64| 2.69, 2.65| C_H 1.26      |
| 34       | Thr     | 8.13     | 4.31| 4.25     | C_H 1.62, C_H 0.95, 0.87 |
| 35       | Leu     | 8.40     | 4.35| 1.68, 1.68|               |
| 36       | Ala     | 7.96     | 4.10| C_H 1.34 |               |

Fig. 3. A, d_{NN} fingerprint region in the NOESY spectrum with pulse-field gradient water suppression at pH 5.6 and 4 °C. Mixing time was 300 ms. Connectivities are shown from residue 18 to 27 only. B, d_{NN} connectivities are likewise illustrated. Amino acids are shown by single letter code.
i + 3) or (i, i + 4) charge-stabilized hydrogen bond (Asp⁶–Arg⁶ and Glu²²–Arg²⁵). The exchangeable N resonances of Arg⁶ and Arg²⁵ were shifted downfield as characteristic of hydrogen bonding; the latter Hₑ exhibited an NOE to a β proton of Glu²².

Inspection of the amino acid sequence suggests that the distribution of negative and positive charges in each helix is aligned with opposite orientation to the helical dipole moment. The assignment of the major helical regions was confirmed by corresponding strings of δNN NOEs (Fig. 3B). A summary of sequential and medium-range NOEs and coupling constants is given in Fig. 4. The pattern of Hα chemical shifts as evaluated by a chemical shift index is also consistent with the assigned secondary structure (Fig. 5). Although initial analyses were consistent with the possibility of higher order structure, more thorough analysis indicated that the range defined by the random coil value ±0.1 ppm. According to statistical data, a grouping of four or more –1 uninterrupted by a +1 indicates a helix and a grouping of four or more +1 uninterrupted by a –1 indicates β strand. All other regions are designated as coil. The cylinder represents the helical region. The dotted cylinder is a potential helix at the C terminus (21).

### Table II

| Residue | J (Hz) |
|---------|--------|
| 1       | 6.36   |
| 2       | 6.40   |
| 3       | 6.49   |
| 4       | 6.53   |
| 6       | 7.26   |
| 10      | 9.30   |
| 11      | 5.66   |
| 12      | 12.20  |
| 14      | 10.18  |
| 15      | 12.04  |
| 16      | 11.26  |
| 17      | 7.02   |
| 18      | 5.31   |
| 19      | 6.30   |
| 21      | 5.66   |
| 22      | 5.58   |
| 23      | 5.41   |
| 24      | 5.69   |
| 25      | 6.92   |
| 29      | 7.04   |
| 30      | 7.03   |
| 31      | 7.90   |
| 32      | 8.20   |
| 34      | 5.51   |

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Peptide E is monomeric in solution. The lack of long-range NOEs in peptide A suggested that the two helices exist independently. However, in previous studies, peptide E, which corresponds to the C-terminal helix (Fig. 1), appeared to be less helical at low concentrations, suggesting that it might dimerize. To re-examine this issue, the secondary structure of peptide E at concentrations ranging from 3 to 100 μM was examined using CD spectroscopy. At all concentrations, the CD spectra of peptide E were nearly identical (data not shown), suggesting that peptide E is monomeric at these concentrations. To further examine the possibility of dimerization, analytical ultracentrifugation experiments were performed. The

![Chemical shift index plotted for Hₐ resonance assignments.](image1)

![Analytical ultracentrifugation of peptide E.](image2)
Activity—structure-activity relationships in the C terminus of Pol.

Fewer long-chain DNA products were formed in the presence of Pol and UL42. A poly(dA)/oligo(dT)15 template/primer was tested for their ability to inhibit long-chain DNA synthesis by variants of peptide E, in which each nonalanine residue was numbered relative to the peptide A sequence, had activities similar to that of peptide E. Mutants T24A, R25A, and D33A were only moderately impaired in their ability to inhibit processivity with IC$_{50}$ values 3–7-fold greater than that of peptide E. Mutants L35A (data not shown) and M27A (Fig. 7B) had even less activity, exhibiting 50 and 40% inhibition at 100 μM, respectively. The most impaired mutants were T20A, E22A, H29A, R30A, and F32A, which exhibited less than 20% inhibition at 100 μM. None of the peptide mutants inhibited Pol catalytic activity in the absence of UL42 (data not shown), demonstrating their specificity for UL42-mediated DNA synthesis.

CD spectroscopy was performed in order to determine if the peptides with the least inhibitory activity were altered structurally. As shown in Fig. 8, wavelength scans of the mutants were characteristic of helical peptides with minima at 222 and 205 nm and a maximum at 190 nm. Mutant F32A had a minimum at 222 nm similar to that of peptide E. Mutants T20A and E22A had substantially lower ellipticity at 222 nm than the other peptides, suggesting a loss of helical content. Therefore, the lack of inhibitory activity of these two mutants may be at least in part due to alterations in structure. Although mutant F32A had a minimum at 222 nm similar to that of peptide E, it had a much deeper minimum at 205 nm, perhaps signifying a loss of helicity in this peptide as well. However, the CD spectra of the H29A and R30A mutants were nearly indistinguishable from that of peptide E, suggesting that Arg$^{29}$ and His$^{30}$ may be directly involved in binding.

**TABLE III**

| Peptide | IC$_{50}$ $^a$ |
|---------|----------------|
| A       | 2.8 ± 1.4      |
| E       | 11 ± 5         |
| T20A    | >100           |
| E22A    | >100           |
| E23A    | 21 ± 8         |
| T24A    | 77 ± 30        |
| R25A    | 40 ± 13        |
| R26A    | 14 ± 9         |
| M27A    | >100           |
| L28A    | 18 ± 6         |
| H29A    | >100           |
| R30A    | >100           |
| F32A    | >100           |
| D33A    | 50 ± 13        |
| T34A    | 10 ± 5         |
| L35A    | 97 ± 5         |

$^a$ Values are means ± S.D. of 3–6 determinations.

Our initial studies focused on peptide A, which spans the C-terminal 36 residues of Pol. This peptide was monomeric and partially ordered at low temperature. Although its folding was not cooperative, NMR spectroscopy defined helical elements...
comprising residues 2–6 and 18–28. The helical propensity of the C-terminal segment (residues 29–36) is indicated by specific \((i, i + 4)\) NOEs between residues 29 and 35 and between residues 28 and 32 and corroborated by trends in \(^1\)H, chemical shifts. The helical propensity of peptide A is thus greater than that inferred from its CD spectrum. It is likely that structural fluctuations within the nascent helices (especially in the residue 29–36 segment) attenuate helix-sensitive CD bands but nonetheless give non-negligible NOEs. This apparent discrepancy reflects the difference in the physics of time averaging between the two techniques and has been previously noted in studies of peptides (18). The incremental and noncooperative unfolding of peptide A contrasts with the highly cooperative and concentration-dependent thermal unfolding of a coiled-coiled dimer (19). Unlike peptide A, the latter also exhibited extensive helix-helix contacts to define a nonpolar interface.

Structure Activity Relationships—Peptide E, which corresponds to the C-terminal helix of peptide A, acts as a monomer and is only slightly less potent an inhibitor of long-chain DNA synthesis than peptide A. The binding of both peptides to UL42 as measured by isothermal titration calorimetry roughly corresponds to the C-terminal helix of peptide A, acts as a monomer and concentration-dependent thermal unfolding of a coiled-coiled dimer (19). Unlike peptide A, the latter also exhibited extensive helix-helix contacts to define a nonpolar interface.

FIG. 8. CD spectra of peptide E mutants. Wavelength scans of peptides in 10 mM potassium fluoride were recorded at 1-nm intervals with a 5-s averaging time, and 5–10 scans were averaged. Peptide concentrations were determined by quantitative amino acid analysis. ○, peptide E; ○, T20A; □, E22A; ▲, R30A, △, H29A; ▲, F32A. MRE, mean residue ellipticity.

directly involved in binding. Together, our findings suggest that the least ordered helical segment corresponding to residues 29–36 is the most critical region for UL42 binding.

Preliminary data from x-ray crystallography studies indicate that peptide A folds into a helix-loop-helix structure when bound to UL42 with the second helix extending to the end of the molecule. Therefore, peptide A binding appears to involve complex-dependent stabilization of the C-terminal helix. This interaction, which is unlike a coiled-coil, may be similar to the assembly of the CREB-CBP transcriptional activator-coactivator complex. Binding of CREB is mediated by a discrete phosphopeptide motif, designated pKID, to the KIX domain of CBP. Similar to peptide A, isolated pKID peptides are monomeric and only partially ordered with an N-terminal α-helix that is observed in aqueous solution and a C-terminal α-helix that is only apparent in trifluoroethanol at 4 °C (18). Upon binding to KIX, a pKID peptide adopts a well ordered helix-turn-helix structure and defines a protein-protein interface unrelated to the coiled-coil (20). The extent of engagement of the two peptide helices correlates inversely with the extent of helical propensity; the intrinsically less stable C-terminal helix is stabilized in the complex by more extensive intermolecular contacts.

Implications for Drug Development—Although the disruption of specific protein-protein contacts is a promising strategy for drug development, the nature of these interactions can make this goal impractical. Many protein-protein interactions involve large surfaces or multiple contacts, making it unlikely that a single small molecule could interfere with them. Our studies identify aspects of the subunit interface of Pol that appear to be important for its contact with UL42. Only a very small surface of Pol appears to be required for UL42 binding, and single amino acid changes drastically reduce inhibitory activity without affecting secondary structure. These findings suggest that the association of HSV Pol and UL42 could be disrupted by a relatively small molecule. Identification of smaller peptides that bind to UL42 or the C terminus of Pol could provide new lead compounds for further development. These approaches can also be applied to the discovery of new drugs for the treatment of other human herpesviruses.

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