Biochemistry. In the article "Conformational changes induced in herpes simplex virus DNA polymerase upon DNA binding" by Klaus Weishart, Alice A. Kuo, George R. Painter, Lois L. Wright, Phillip A. Furman, and Donald M. Coen, which appeared in number 3, February 1, 1993, of *Proc. Natl. Acad. Sci. USA* (90, 1028–1032), the 43-kDa bands in lanes 6 and 7 of Fig. 4 were not dark enough to be readily visible. The authors have provided a darker version of that figure, which is shown here with its legend.

![Fig. 4.](image)

**Fig. 4.** Identification of proteolytic products. Ten micrograms of HSV Pol was digested in separate reactions in the absence (lane 1) or presence (lanes 2–7) of 25 μg of (dA)₉₀₀p(dT)₁₀–₁₂ at a protease/polymerase ratio of 1:250. Reaction products were separated by SDS/13% PAGE and then transferred to an Immobilon-P membrane. The membrane was cut in strips. One strip contained reaction mixtures without (lane 1) and with (lane 2) DNA and was stained with Coomassie blue. The other strips contained exclusively reaction mixtures with DNA. They were probed with antibodies EX6 (lane 3), EX1051 (lane 4), EX3 (lane 5), BGG4 (lane 6), and PP5 (lane 7). Fragments are identified at left by their molecular sizes (kDa). The position of the 43-kDa product is indicated by an arrowhead.

Cell Biology. In the article "Tumor necrosis factor α is an autocrine growth regulator during macrophage differentiation" by Alice L. Witsell and Lawrence B. Schook, which appeared in number 10, May 1992, of *Proc. Natl. Acad. Sci. USA* (89, 4754–4758), the authors request that the following correction be noted. In the second section (Antisense Oligomers) of Materials and Methods (p. 4755), the TNF-α and IL-1β antisense oligomers were 5'-GGATCATGCTTCTGTG-3' and 5'-TCAGGAACAGTTGCCAT-3' and were targeted to serine and methionine residues, respectively.
Conformational changes induced in herpes simplex virus DNA polymerase upon DNA binding

(fluorescence spectroscopy/limited proteolysis/polymerase mechanisms/antiviral drugs)

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ABSTRACT Herpesvirus DNA polymerases are prototypes for α-like DNA polymerases and important targets for antitherpesvirus drugs. We have investigated changes in the catalytic subunit of herpes simplex virus (HSV) polymerase following DNA binding by using the techniques of endogeneous fluorescence quenching and limited proteolysis. The fluorescence studies revealed a reduction in the rate of quenching by acrylamide in the presence of DNA without changes in the wavelength of the emission peak or in the lifetime of the fluorophore, consistent with the possibility of conformational changes. Strikingly, the proteolysis studies revealed that binding to a variety of natural and synthetic DNA and RNA molecules induced the appearance of a new cleavage site for trypsin near residue 1660 of the protein and increased cleavage by trypsin near the center of the protein. The extent of these cleavages correlated with the affinity of the polymerase for these ligands. These data provide strong evidence that binding to nucleic acid polymers induces substantial localized conformational changes in the polymerase. The locations of enhanced tryptic cleavage near sites implicated in substrate recognition and interaction with a processivity factor suggest that the conformational changes are important for catalysis and processivity of this prototype α-like DNA polymerase. Inhibition of these changes may provide a mechanism for antitherpesvirus drugs.

A prerequisite of DNA replication is the interaction of DNA polymerase with DNA. Much of what has been learned about DNA polymerases in general, and their binding to DNA in particular, is derived from studies of Escherichia coli DNA polymerase I (Pol I). X-ray crystallography of the large proteolytic (Klenow) fragment of Pol I has shown that its C-terminal polymerase domain possesses a binding cleft that could accommodate a double-stranded B-DNA helix, whereas the N-terminal 3'-5' exonuclease domain can independently bind single-stranded DNA (1-3). These crystallographic analyses, combined with genetic and biochemical studies, have greatly enhanced our understanding of this enzyme (4-10). However, little has been published regarding changes in Pol I upon DNA binding other than a few changes in side chains in contact with single-stranded DNA (3).

Aside from Pol I and its relatives, the other major class of DNA polymerases is the α-like class (also known as class B), which includes the eukaryotic replicative polymerases (11, 12). The DNA polymerase encoded by herpes simplex virus (HSV Pol) serves as an excellent prototype of the α-like polymerases in that it is amenable to genetic, pharmacological, and biochemical study, and much information has been obtained regarding functional sites on this molecule (re-viewed in ref. 13). Herpesviruses are important pathogens, especially in immunocompromised patients such as those with AIDS, and herpesvirus polymerases such as HSV Pol are important targets for antiviral drugs such as acyclovir (reviewed in ref. 14). Information about these enzymes may aid in further drug development. Little is known about the mechanisms involved in DNA binding of α-like polymerases such as HSV Pol, but sequence homology and modeling exercises have suggested that DNA binding by diverse polymerases might occur in a fashion similar to that of Pol I (15).

Support for this idea has come from the recent crystal structure of human immunodeficiency virus (HIV) reverse transcriptase (16), which like Pol I has a large cleft that could accommodate double-stranded nucleic acids. Conformational changes in proteins are frequently critical for function and/or regulation (17); for example, a conformational change is important in the mechanism of Pol I fidelity (8). We have looked for conformational changes in HSV Pol upon binding to DNA, using spectroscopic and proteolytic techniques. The spectroscopic approach relies upon the endogenous fluorescence of HSV Pol, which is due to the presence of nine tryptophan residues (18, 19), and the use of acrylamide, which quenches tryptophan fluorescence of proteins by physical contact with an excited indole ring. Acrylamide quenching depends on the accessibility of the tryptophans to the acrylamide; therefore, acrylamide quenching can be a useful indicator of protein conformational changes (20).

Susceptibility to proteolysis can also yield valuable information about conformational changes, again by monitoring differences in accessibility of the protein to a probe, in this case a protease (21). Especially revealing are instances when protease cleavage is enhanced, indicating increased accessibility. If sites of cleavage are known, conformational changes can be mapped to certain regions within the protein. The spectroscopic and the proteolytic approaches provided strong evidence for conformational changes in HSV Pol following binding to DNA. The proteolysis studies mapped the locations of these changes near sites implicated as critical for catalysis and processivity.

MATERIALS AND METHODS

Materials. The catalytic subunit of HSV Pol was purified from recombinant baculovirus BP58-infected Sf9 cells (22) by a procedure to be described elsewhere (K.W., A.A.K., C. B. C. Hwang, and D.M.C., unpublished work). Antibod-

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Abbreviations: Pol I, E. coli DNA polymerase I; HSV Pol, herpes simplex virus DNA polymerase; HIV, human immunodeficiency virus; RF, replicative form.

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ies EX6, EX1051, EX3, BGG4, and PPS have been described (23, 24). Activated calf thymus DNA (Sigma) was purified (25) to remove extraneous fluorescent proteins. Acrylamide (99% purity) was obtained from Sigma. (tA)3000p(rP)700, (dA)3000p(rP)700, (tA)2000, (rA)2000, and single-stranded and replicative-form (RF) M13mp18 DNAs were purchased from Pharmacia LKB. Endoproteinase-GluC (Staphylococcus aureus V8 protease), sequencing-grade trypsin, phenylmethylsulfonyl fluoride, and leupeptin were obtained from Boehringer Mannheim. HindIII-digested λ DNA was purchased from BRL. Protein molecular weight standards were obtained from Bio-Rad. Immobilon-P and Problot poly(vinylidene difluoride) membranes were obtained from Millipore and Applied Biosystems, respectively.

**Fluorescence Spectroscopic Analysis.** Theory. Quenching of a single tryptophan residue is described by the Stern–Volmer equation (26):

$$F_0/F = 1 + k_{q}\tau_0[Q] = 1 + k_{SV}[Q],$$

where $F_0$ and $F$ are the fluorescence intensities in the absence and presence of quencher, respectively; $k_{q}$ is the bimolecular quenching constant; $\tau_0$ is the lifetime of the fluorophore in the absence of quencher; $k_{SV}$ is the Stern–Volmer quenching constant, and $[Q]$ is the concentration of quencher. For proteins with multiple tryptophan residues, the observed $K_{SV}$ ($k_{SV}(\text{eff})$) may be considered to be a collisional quenching constant if it increases with increasing temperature. If the plot of $F_0/F$ vs. $[Q]$ is linear, which indicates a single class of fluorophores all equally accessible to quencher, then $k_{SV}(\text{eff}) = k_{q}(\text{eff})\tau_0$ (27). $k_{eff}$ is an estimate of the average exposure of fluorescent tryptophan residues to acrylamide. To determine values of $k_{eff}$ from $K_{SV}(\text{eff})$, the average lifetime, $\tau_0$, of the fluorophores must be measured both in the absence and in the presence of bound DNA.

**Experimental.** Two and two-tenths milliliters of a 2.0-μg/ml solution of HSV Pol with or without activated calf thymus DNA (30 μg/ml) in 50 mM Tris-HCl, pH 7.6/5 mM MgCl2 was titrated with aliquots of a 4 M solution of acrylamide at 25°C or 37°C. The change in fluorescence intensity was monitored at 340 nm, the $\lambda_{max}$ of HSV Pol. The titrations were performed in a quartz cuvette in the thermostat sample chamber of a Perkin–Elmer LS-50 fluorometer. With activated calf thymus DNA, at 30 μg/ml, all HSV Pol molecules are bound (unpublished data). Values of $K_{SV}(\text{eff})$ were determined from a fit of Eq. 1 to the data and are the average of at least two independent experiments. All fluorescence measurements were corrected for inner filter effects (which were minimized by excitation at 290 nm). Fluorescence lifetimes were measured by using a frequency-domain apparatus at the Center for Fluorescence Spectroscopy at the University of Maryland at Baltimore School of Medicine (28). The samples contained HSV Pol at 2.1 μg/ml in the absence or presence of activated calf thymus DNA at 30 μg/ml in 350 μl of 30 mM Tris-HCl, pH 7.6. The output of an Ar+-pumped Rhodamine 6G dye laser was frequency-doubled to obtain the 295-nm excitation light, and fluorescence was observed through a 340-nm interference filter by using "magic angle" polarizer conditions. Average lifetimes were determined from a nonlinear least-squares regression analysis.

**Proteolytic Analysis of HSV Pol.** Trypsin was dissolved in 1 mM HCl. *S. aureus* V8 protease was dissolved to 1 mg/ml in water and further diluted in 10 mM KP (pH 8). HSV Pol was incubated with the indicated nucleic acid polymer or mock-incubated for 10 min at room temperature in the appropriate buffer (50 mM Tris-HCl for trypsin) or 50 mM KP (pH 7.6) for V8 protease prior to proteolysis. The proteolytic reactions were initiated by adding the stated amount of protease. After 30 min at room temperature, trypsin digestion was terminated by the addition of phenylmethylsulfonyl fluoride at 40 μM/ml for 2 min on ice, whereas V8 protease digestion was terminated by leupeptin at 0.5 μg/ml. Samples were subjected to SDS/PAGE (29). Gels were either Coomassie- or silver-stained (30) or proteins were transferred to Immobilon-P membranes (31). Membranes were probed with antibodies using the Protein Western blot alkaline phosphatase system (Promega) for detection or were directly stained in 0.1% Coomassie brilliant blue/50% methanol. Proteolytic fragments were prepared for sequencing as outlined by Matsudaira (32) and subjected to automated peptide sequencing at the Harvard University Microchemistry Facility.

**Filter DNA-Binding Assay.** Filter DNA-binding assays (33) were performed by incubating 2 μmol of HSV Pol with 15 fmol (1 ng) of 5'-end-labeled 96-bp EcoRI-BamHI fragment (10,000 cpm/fmol) in 100 μl of buffer A (50 mM Tris-HCl, pH 7.6/0.5 mM dithiothreitol/10% glycerol/2.5 mM MgCl2). After 20 min the reaction mixture was diluted in buffer A to 1 ml and passed through nitrocellulose filters equilibrated in buffer A. Radioactivity that bound was quantified by liquid scintillation counting. Under these conditions, half of the input DNA was retained on the filter. Competition experiments were performed by adding different amounts of unlabelled nucleic acid ligands to the reaction mix and determining the amount of ligand sufficient to displace 50% of the radioactive DNA by interpolation. When single-stranded nucleic acids were used for competition, MgCl2 was omitted from buffer A.

**RESULTS**

**Probing Conformational Changes in HSV Pol by Fluorometric Measurements.** The background corrected fluorescence emission spectrum of HSV Pol revealed an emission maximum ($\lambda_{max} = 340$ nm) that was blue-shifted relative to that of free l-tryptophan ($\lambda_{max} = 350$ nm) under the same conditions. This can be ascribed to shielding of one or more of the tryptophan residues from the aqueous phase by the three-dimensional structure of the enzyme (20). Addition of activated calf thymus DNA at 30 μg/ml decreased the quantum yield of HSV Pol by 30% without a change in $\lambda_{max}$ (data not shown). No change in $\lambda_{max}$ was observed after addition of acrylamide. The values of $K_{SV}(\text{eff})$ determined from the linear (coefficient of determination, $r^2 > 0.997$) Stern–Volmer plots (Eq. 1; Fig. 1) decreased from 6.63 ± 0.26 M⁻¹ at 37°C to 5.24 ± 0.23 M⁻¹ at 25°C and to 3.83 ± 0.18 M⁻¹ at 10°C.
at 25°C in the presence of activated calf thymus DNA at 30 µg/ml. The average fluorescence lifetimes of the protein free and bound to activated calf thymus DNA at 25°C were not different ($\tau_0 = 4.0$ ns), yielding values of 1.31 ± 0.06 and 0.96 ± 0.05 × 10⁸ M⁻¹s⁻¹, respectively, for $k_\text{eff}$ demonstrating a decrease in accessibility of the tryptophan residues to the quencher, without a change in $\lambda_{\text{max}}$.

**Changes in Trypsin Cleavage Patterns Following DNA Binding.** We probed for conformational changes in HSV Pol by looking for changes in cleavage by trypsin upon binding of various nucleic acid ligands. Limited trypsin cleavage of HSV Pol not bound to DNA yielded four major stable fragments: 80- and 72-kDa fragments that start at amino acid 42, a 50-kDa fragment that starts at amino acid 693, and a 12-kDa fragment that starts at amino acid 1120 (Fig. 2 and K.W., A.A.K., C. B. C. Hwang, and D.M.C., unpublished work). An example of this cleavage pattern is shown in Fig. 3A, lane 3. When HSV Pol was incubated with (dA)₅₀₀-p(dT)₁₀₁₂ before trypsin digestion, however, the pattern of fragments changed, dependent upon the concentration of ligand (Fig. 3A, lanes 4-7). With increasing DNA concentration, the N-terminal 80-kDa fragment decreased, as did the 50-kDa C-terminal fragment. In contrast, the amount of the 72-kDa N-terminal fragment increased and a new fragment of 43 kDa was observed. The amount of 12-kDa fragment remained relatively unchanged (the modest decrease seen in Fig. 3A was not reproducible). We also did not observe any meaningful change in the pattern of S. aureus V8 protease digestion of HSV Pol upon DNA binding (data not shown).

**Correlation of Changes of Cleavage Pattern with Binding Affinity.** Various nucleic acid polymers were evaluated for their abilities to cause this change in digestion pattern.

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**Fig. 2.** Cleavage map of trypsin on HSV Pol. The top line shows the locations of regions of sequence homology and function on the HSV Pol polypeptide. The second line provides a scale relating polypeptide size in kilodaltons ("kd") and amino acid (aa) residue number. Below the scale line are open bars indicating locations of regions specific for the antibodies EX6, EX1051, EX3, BGG4, and PP5 (23, 24). Numbers designate amino acid residues in the polypeptide. The lines below show the cleavage map of trypsin. Thick vertical bars represent cleavage sites. The amino acid residues corresponding to N-terminal cleavage positions of the 80-, 72-, 50-, 43-, and 12-kDa fragments are indicated; locations of the other sites are approximate. Arrowheads indicate the cleavage sites that increase in accessibility, in the central and C-terminal regions of the enzyme, following DNA binding. Locations of fragments are indicated by the horizontal lines with numbers that represent their molecular mass. (Due to uncertainties in size determinations, sizes of fragments do not necessarily add up to the size of full-length HSV Pol.)

**Fig. 3.** Proteolytic analysis in the presence or absence of various nucleic acids. (A) Four micrograms of HSV Pol was digested in the absence (lane 3) or presence of 0.01 µg (lane 4), 0.1 µg (lane 5), 1 µg (lane 6), or 10 µg (lane 7) of (dA)₅₀₀-p(dT)₁₀₁₂ at a protease/polymerase ratio of 1:250, and the products were analyzed by SDS/PAGE and Coomassie blue staining. A mock-digested control, containing 10 µg of the nucleic acid (U, lane 2), and molecular size markers (M, lane 1) were analyzed in parallel. (B) Four micrograms of HSV Pol was digested in the absence (lane 3) or presence of 5 µg (lanes 4-9 and 11) or 50 µg (lane 10) of the indicated nucleic acid ligand. CT, calf thymus; ss, single-stranded. Proteolytic products were separated by SDS/13% PAGE and the gel was stained with Coomassie blue. A mock-digested sample (lane 2) and markers (lane 1) were analyzed in parallel. Below the gel is shown the concentration (ng/µl) of each nucleic acid that was able to inhibit 50% (IC₅₀) of the binding of HSV Pol to 1 ng of a 5'-end-labeled 96-bp EcoRI-BamHI fragment. For both A and B, molecular sizes (kDa) of markers are indicated at left and the proteolytic fragments are identified by their molecular sizes at right.

(dA)₂₀₀, (rA)₂₀₀, (dA)₅₀₀-p(dT)₁₀₁₂, (rA)₅₀₀-p(dT)₁₀₁₂, activated calf thymus DNA, single-stranded M13mp18, RF M13mp18, and λ HindIII fragments all gave rise to qualitatively similar changes (Fig. 3B). However, the extent of change depended greatly on the nucleic acid used. For example, 5 µg of single-stranded M13mp18 DNA led to a complete disappearance of the 80-kDa fragment and the appearance of substantial amounts of the new 43-kDa fragment (lane 9), whereas 50 µg of RF (closed circular double-stranded) M13mp18 DNA led to very little change in pattern (lane 10), compared with no DNA. To determine whether this correlated with the binding affinity of HSV Pol for these nucleic acids, the ability of each ligand to compete with 1 ng of a labeled 96-bp EcoRI-BamHI fragment was measured. The concentration of each ligand that could block 50% of the binding of the 96-bp fragment is shown at the bottom of Fig. 3B. There was a strong correlation between binding affinity as measured in the competition assay and the extent of change in the digestion pattern.

**Mapping of Sites of Cleavage Following DNA Binding.** To determine the locations of the proteolytic cleavage sites that changed upon binding to DNA, we used region-specific antibodies and peptide sequencing to map the tryptic fragments produced in the presence of DNA. As was true in the absence of DNA (K.W., A.A.K., C. B. C. Hwang, and D.M.C., unpublished work), the N termini of the 80-
Fig. 4. Identification of proteolytic products. Ten micrograms of HSV Pol was digested in separate reactions in the absence (lane 1) or presence (lanes 2–7) of 72-kDa fragment with antibodies EX6 (lane 3), EX1051 (lane 4), EX3 (lane 5), BGG4 (lane 6), and PP5 (lane 7). Fragments are identified at by their molecular sizes (kDa).

72-kDa fragments were amino acid 42, the N terminus of the 50-kDa fragment was amino acid 693, and the N terminus of the 12-kDa fragment was amino acid 1120. As was true in the absence of DNA (data not shown), the 72-kDa fragment reacted with antibody EX1051, but less strongly than did the 80-kDa fragment (Fig. 4). This places the C terminus of the 72-kDa fragment between residues 597 and 685; if the C terminus of the 80-kDa fragment is residue 693, the size difference between the 80- and 72-kDa fragments predicts that DNA binding increases trypsin cleavage near residue 620.

The 43-kDa product that was observed only in the presence of DNA failed to react with EX6, EX1051, or EX3 antiserum but did react with antisera BGG4 and PP5 (Fig. 4). This result showed that the 43-kDa product lay within the C-terminal half of HSV Pol (Fig. 2). This was confirmed by peptide sequencing, which revealed that the N terminus of the 43-kDa fragment, like that of the 50-kDa fragment, was residue 693. This location and the size of the fragment predict a C terminus near residue 1060.

**DISCUSSION**

**Changes in Fluorescence Quenching Suggest a Conformational Change.** Acrylamide quenches the fluorescence of tryptophan(s) in HSV Pol by a collisional process as evidenced by the temperature dependence of the quenching constants, $k_{sv}$(eff) (Fig. 1). The linear Stern-Volmer plots indicate both that the fluorescence tryptophan residues differ only slightly in accessibility and that $k_{sv}$(eff) is a quantitative measure of the accessibility of the tryptophans to quencher. The rate of acrylamide quenching was slower in the presence of DNA; thus, the accessibility of the tryptophans is decreased. There are two possible explanations for this decrease due to DNA binding: (i) the fluorescing residues are shielded from collision with acrylamide directly by the binding of DNA or (ii) the binding causes a conformational change resulting in decreased exposure of some of the fluorescing residues (20). Arguing against the first explanation is our finding that addition of DNA decreased the quantum yield of HSV Pol but did not affect the fluorescence lifetime. This indicates that DNA is bound by a static process—i.e., ground-state binding (27)—such that tryptophans involved in DNA binding are no longer fluorescent. Thus, any remaining fluorescence would be due to tryptophans some distance from the DNA. Therefore, the decrease in the accessibility of these tryptophan residues to quencher upon DNA binding suggests conformational changes in the enzyme (probably local rather than global, since $\Delta_{max}$ remained constant and the Stern-Volmer plot remained linear). This would be analogous to the report of Philips et al. (34), who used acrylamide quenching to detect conformational changes in human adenosine deaminase upon ground-state inhibitor binding.

**Changes in Trypsin Cleavage Confirm the Conformational Change and Localize It.** It is difficult to imagine how DNA binding could increase the accessibility of trypsin to a given cleavage site without a conformational change in the protein. Indeed, one of the sites observed in the presence of DNA, which led to the 43-kDa fragment, was not observed, at all in its absence, which indicates an enormous increase in the accessibility of that site.

The proteolysis studies indicate where certain conformational changes occur. The results make clear that DNA binding does not cause a global change in the conformation of HSV Pol, because accessibility to the cleavage site at gives rise to the 12-kDa fragment did not meaningfully change and because no change was observed in the pattern of digestion by V8 protease. The tryptic cleavage sites whose accessibility increases map near residues 620 and 1060. This indicates that conformational changes upon DNA binding occur near these residues but does not rule out the possibility that changes occur elsewhere in the molecule.

Little has been published regarding conformational changes in DNA polymerases upon binding to DNA. DNA binding prevents a dual activation of catalytic activity of Pol I during the preparation of Kleenoprotein fragment (35); however, this may be due to protection of cleavage sites by DNA rather than conformational changes.

**Implications for DNA Binding.** The locations of the sites of trypsin cleavage that change upon DNA binding suggest that DNA binds within the C-terminal half of HSV Pol. This suggestion, which is attractive because there are data that implicate this region in polymerase activity (reviewed in refs. 13 and 14), has been confirmed (K.W., A.A.K., and D.M.C., unpublished results). One would also expect nucleic acid contacts within the N-terminal half of the molecule, which has been hypothesized to harbor the RNase H and 3'-5' exonuclease activities (22, 36–38); indeed, we have detected DNA binding by this half of the molecule as well (K.W., A.A.K., and D.M.C., unpublished results).

It has been hypothesized (15, 36, 39) that α-like DNA polymerases such as HSV Pol might be analogous to the Kleenoprotein fragment of E. coli Pol I, in which the C-terminal polymerase domain binds the double-stranded region of primer-template while the C-terminal 3'-5' exonuclease domain can bind single-stranded DNA independently. If this hypothesis were correct, one might predict single-stranded DNA to elicit different conformational changes than DNAs containing double-stranded regions. However, not only did single-stranded DNAs elicit similar changes to double-stranded DNAs, but they did so more efficiently, concomitant with higher affinities for HSV Pol (Fig. 3B). This would be consistent with evidence suggesting that 3'-5' exonuclease and polymerase domains of HSV Pol and other α-like DNA polymerases are not as structurally and functionally independent as those of Pol I and its relatives (refs. 40-42 and K.W., A.A.K., C. B. C. Hwang, and D.M.C., unpublished work).

We have also found that RNA-containing nucleic acids bound to HSV Pol (Fig. 3B and unpublished results). This in itself is not surprising given the intrinsic RNase H activity of HSV Pol (22, 37). What was somewhat surprising was that RNA-containing molecules, even poly(rA), elicited changes that were indistinguishable from those elicited by DNA. We can imagine two possible interpretations of this finding: (i) either binding of RNA to the RNase H site of HSV Pol, which is thought to be in the N-terminal half of the molecule, elicits a conformational change in the C-terminal half of the molecule, suggesting functional interdependence of different portions of the enzyme, or (ii) RNA can bind directly to the C-terminal.
half of HSV Pol, which may have interesting implications for polymerase primer–template recognition.

**Implications for Polymerase Mechanisms and Antiviral Drugs.** The site in the center of HSV Pol whose accessibility increases upon DNA binding is near or within regions A and II of sequence homology shared among α-like DNA polymerases. Mutations that cause codon and/or analogos sensitivity for nucleotide analogs map within these regions, implicating them directly or indirectly in dNTP recognition (reviewed in refs. 13 and 14). Our best guess for the location of the site is within region A (residues 577–637), which is shared among certain viral and cellular DNA polymerases (14, 43). However, from the limited resolution of our analysis, the site may be adjacent to region II (residues 694–736), which has been proposed to directly interact with dNTPs (43) on the basis of several altered drug-sensitivity mutations mapping therein. This region also contains a highly conserved aspartic residue that is thought to be involved in catalysis in diverse polymerases (15, 16). The results invite speculation that DNA binding changes of HSV Pol binds dNTPs in conjunction with primer–template, thereby increasing the selectivity of incorporation.

The site near residue 1060 is adjacent to a separable C-terminal domain (K.W., A.A.K., C. B. C. Hwang, and D.M.C., unpublished results) of HSV Pol, defined in part by the 12-kDa tryptic fragment which starts at residue 1120. This domain interacts with the processivity factor UL42 (refs. 44 and 45; K.W., A.A.K., and D.M.C., unpublished results). It has been proposed that UL42 increases the processivity of HSV Pol by increasing binding to primer–template (46). The identification of a conformational change adjacent to this region suggests that DNA binding may alter the interaction of the HSV Pol catalytic subunit with UL42. This change could increase the affinity of the heterodimer for primer–template further and/or could wrap part of the catalytic subunit around the DNA to permit the heterodimer to form a "sliding clamp" that can translocate efficiently along the DNA without falling off. This would be similar to a model proposed for E. coli Pol I in which an unstructured flexible region would close on DNA once it is bound to the cleft in the C-terminal domain (5), for which there is now x-ray crystallographic evidence (L. Beese and T. Steitz, personal communication). In this regard, it is interesting that residues 1080–1140 are predicted to be hydrophilic and flexible (23, 39).

Alternatively, this C-terminal domain or the regions near the center of the protein may move at certain stages of the catalytic cycle, as has been proposed for the "thumb" subdomains of Pol I and HIV reverse transcriptase (15, 16). Indeed, the anti-HIV drug nevirapine, which binds to a site between different subdomains of HIV reverse transcriptase, has been proposed to act by preventing such a conformational change (16). We suspect that the conformational changes we have detected in HSV Pol are critical for the functioning of this enzyme and can envision the development of antiviral drugs that could act by binding to herpesvirus DNA polymerases and interfering with such changes.

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