The Positively Charged Surface of Herpes Simplex Virus UL42 Mediates DNA Binding

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Herpes simplex virus DNA polymerase is a heterodimer composed of UL30, a catalytic subunit, and UL42, a processivity subunit. Mutations that decrease DNA binding by UL42 decrease long chain DNA synthesis by the polymerase. The crystal structure of UL42 bound to the C terminus of UL30 revealed an extensive positively charged surface (“back face”). We tested two hypotheses, 1) the C terminus of UL30 affects DNA binding and 2) the positively charged back face mediates DNA binding. Addressing the first hypothesis, we found that the presence of a peptide corresponding to the UL30 C terminus did not result in altered binding of UL42 to DNA. Addressing the second hypothesis, previous work showed that substitution of four conserved arginine residues on the basic face with alanines resulted in decreased DNA affinity. We tested the affinities for DNA and the stimulation of long chain DNA synthesis of mutants in which the four conserved arginine residues were substituted individually or together with lysines and also a mutant in which a conserved glutamine residue was substituted with an arginine to increase positive charge on the back face. We also engineered cysteines onto this surface to permit disulfide cross-linking studies. Last, we assayed the effects of ionic strength on DNA binding by UL42 to estimate the number of ions released upon binding. Our results taken together strongly suggest that the basic back face of UL42 contacts DNA and that positive charge on this surface is important for this interaction.

Most replicative DNA polymerases depend on accessory proteins known as processivity factors to achieve prolonged association with DNA. In this way polymerases are able to synthesize long stretches of DNA before dissociating from their templates. The most studied processivity factors include proliferating nuclear antigen (PCNA)3 from eukaryotes and archaebacteria (1, 2), the β-subunit from Escherichia coli (3), and gp45 from T4 and RB69 bacteriophage (4, 5). These ring shaped proteins, also known as “sliding clamps,” cannot bind DNA on their own and require protein complexes called clamp loaders to be loaded onto DNA as toroidal homomultimers in an ATP-dependent manner (6). They are then able to physically tether the catalytic subunit of polymerase, thus ensuring its processivity (7–9).

The DNA polymerase encoded by herpes simplex virus (HSV) is a heterodimer composed of a catalytic subunit, UL30, and a processivity subunit, UL42 (10–12). UL42 interacts with the C terminus of UL30, increases affinity of the polymerase for primer/template DNA, and stimulates long chain DNA synthesis (13–18). In contrast to sliding clamps, UL42 binds DNA directly as a monomer with nanomolar affinity and does not require ATP hydrolysis or accessory proteins for binding (10, 14, 18, 19). Even though UL42 binds DNA tightly, it is able to diffuse on DNA and does not impede UL30 elongation (18, 20).

The crystal structure of UL42 has been solved in complex with the C-terminal 36 residues of UL30 (21). The structural fold of UL42 is remarkably similar to that of a monomer of PCNA even though these two proteins have no obvious sequence homology (21, 22). The structure of UL42 reveals a basic surface on the side opposite the UL30 binding site (back face). Substitution of any of four conserved arginine residues on this face with alanines results in decreased DNA binding affinity (23). These alanine substitution mutants and other mutants that decrease DNA binding affinity of UL42 also decrease long chain DNA synthesis of UL30/UL42 even though these mutants retain wild-type affinity for the C-terminal 36 residues of UL30, suggesting that DNA binding by UL42 is important for processive DNA synthesis (23, 24). These findings, however, did not address whether the importance of the arginine residues for DNA binding is due to their basic charge, whether the basic surface contacts DNA, and, if it does, how many charge-charge interactions are involved in binding. Addressing these issues should aid understanding of how this processivity factor can bind DNA tightly yet diffuse on DNA.

Another mechanism by which processivity factors could modulate their interaction with DNA is by undergoing a conformational change upon binding to interacting proteins.

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2 The on-line version of this article (available at http://www.jbc.org) contains supplemental Table 1.

3 The abbreviations used are: PCNA, proliferating cell nuclear antigen; HSV, herpes simplex virus; HCMV, human cytomegalovirus; MBP, maltose-binding protein; FP, fluorescence polarization.
Indeed, it has been proposed that the interaction of UL42 with UL30 leads to a conformational change that results in increased affinity of the polymerase for DNA (25). We have previously found that the affinity for DNA of UL44, a subunit of human cytomegalovirus (HCMV) DNA polymerase, increases when it interacts with the C terminus of its cognate catalytic subunit, UL54 (26). This increased affinity correlates with differences between the crystal structures of unliganded UL44 and UL44 in complex with a peptide from the C terminus of UL54 (22, 26). In both structures UL44 forms a head-to-head homodimer in the shape of a C clamp. In the peptide-bound structure, however, the C-shaped clamp is more open (26), suggesting a conformational change that increases affinity for DNA. We, therefore, wished to investigate whether the peptide corresponding to the C terminus of UL30 is able to alter the affinity of UL42 for DNA.

To investigate these questions, we used a combination of biochemical, mutational, and cross-linking approaches. Although we found no support for the notion that the C terminus of UL30 affects DNA binding by UL42, our data strongly suggest that UL42 interacts with DNA via its basic back face and further suggests that positive charge on this surface participates in this interaction.

**EXPERIMENTAL PROCEDURES**

**Plasmids**—The pMBP-PP-UL42ΔC340 and MBP-PP-UL42ΔC320 plasmids, which express the N-terminal 340 and 320 residues of UL42, respectively, with a maltose-binding protein (MBP) at the N terminus and a PreScission Protease site in between MBP and UL42 were described previously (21, 23). These proteins retain the biochemical and biological activities of full-length UL42 (27, 28) and will be referred to as wild type below. The MBP-PP-UL42ΔC320 plasmid was used as a starting point to construct plasmids expressing the R51C and R182C mutant proteins. All other plasmids expressing mutant proteins used in this study were constructed from pMBP-PP-UL42ΔC340. Mutations were introduced using the QuikChange mutagenesis kit (Stratagene) according to the manufacturer’s directions. Primer sequences used to create these mutants are listed in supplemental Table 1. All plasmids were sequenced to confirm the presence of the mutation(s) and the absence of undesired mutations.

**Proteins and Peptides**—Wild-type and mutant UL42 proteins were expressed and purified as described previously (23) with minor modifications. Briefly, all UL42 proteins were expressed at room temperature for 3–5 h in *E. coli* BL21(DE3)pLysS cells (Novagen). They were purified first on amylose resin (New England Biolabs) followed by a heparin HiTrap column (GE Healthcare). The proteins were eluted from heparin columns using a 50 mM–1 M NaCl gradient rather than a 10–500 mM NaCl gradient. The wild-type, R51C, and R182C UL42ΔC320 proteins were cleaved with PreScission protease (GE Healthcare) to remove the MBP tag before being purified on heparin column. The 36-residue UL30 peptide and the corresponding Oregon-Green-labeled peptide were synthesized at the Biopolymers Facility of the Department of Biological Chemistry and Molecular Pharmacology (Harvard Medical School). Concentrations of proteins and peptides were determined by amino acid analysis at the Molecular Biology Core Facility at Dana-Farber Cancer Institute.

**DNA Binding Assays**—Filter binding assays to compare DNA binding by wild-type UL42 in the absence or presence of 50 μM peptide corresponding to the C-terminal 36 residues of UL30 or to compare DNA binding by wild-type and mutant UL42 proteins were performed as previously described (18). Briefly, 1 fmol of radiolabeled ~100-bp DNA was incubated with increasing concentrations of either wild-type or mutant UL42. To separate protein-bound DNA from free DNA, samples were passed through nitrocellulose and DE81 filters. Filters were then washed and dried, and radioactivity was measured by liquid scintillation counting. Because the concentration of DNA was much less than the observed *Kd* values, the apparent *Kd* values could be determined by a saturation isotherm analysis in which the data were fit to the equation Fraction DNA bound = ([UL42]/(*Kd* + [UL42])). Best fit plots are presented in the figures.

Filter binding assays to measure the affinity of UL42 for DNA in different ionic environments were performed as previously described for HCMV UL44 (29), except that a 30-bp DNA was used. Calculations of charge-charge interactions were performed using the analyses developed by Record and co-workers (30–32) as previously described (29). Briefly, according to these analyses, when a ligand with positive charges in its DNA-binding site binds to a nucleic acid, Z phosphates are effectively neutralized, releasing the counterions that were previously associated with the phosphates into solution along with ions involved in long-range screening interactions. The theory predicts that in the presence of a monovalent salt MX, the amount of counterion release can be determined from the formula

$$\log [Z] = \frac{1}{2} \log [K_{d,MX}] = -Z \psi \log [M^+],$$

where *ψ* is a constant (0.88 for duplex B-form DNA), and *Kd,MX* is calculated as 1/observed *Kd*. This permits determination of *Z* from the dependence of log *Kobs* on log[M⁺]. To help assess whether anion release meaningfully contributes to the salt effects observed, one can assess the effect of a different monovalent salt and also the effect of a divalent cation such as Mg²⁺ on binding. In this latter case,

$$\log K_{obs}/\log [M^{2+}] = -\varphi [M^{2+}],$$

where *φ* = 0.47 for B-form DNA. In the absence of meaningful anion effects, when the same anion is used, then

$$\log K_{obs}/\log [M^{2+}] = (\varphi/\psi) \log K_{obs}/\log [M^+]$$

should hold, i.e. the ratio of the slopes should be 0.53.

**Measurements of Binding of UL42 to the C Terminals of UL30**—The interactions between wild-type or mutant UL42 proteins and a 36-residue peptide corresponding to the C terminus of UL30 were measured either by isothermal titration calorimetry or by fluorescence polarization (FP), which yield similar affinities for the wild-type UL42-UL30 peptide interaction (27, 33). Isothermal titration calorimetry experiments were performed as described previously (27). Concentrations of UL42 proteins were 2–20 μM, and the concentration of the 36-residue UL30 peptide ranged between 146 and 218 μM. FP assays were performed as described previously with minor modifications (33). Briefly, 1 nM of Oregon Green-labeled 36-residue UL30 peptide was added to increasing concentrations of wild-type or mutant UL42 protein in 96-well plates in a total volume of 80 μl per well. After incubation at room temperature for 20 min, the FP values were determined by using an Analyst plate reader (LJL).
At the Institute of Chemistry and Cell Biology-Longwood Screening Facility (Harvard Medical School). Assays of UL42-mediated Long Chain DNA Synthesis—DNA polymerase assays were performed as described previously (23, 24) using a poly(dA)/oligo(dT) primer-template, radiolabeled dTTP, 200 fmol of UL30, and either 800 or 1200 fmol of UL42. Reaction products were analyzed on alkaline agarose gels that were exposed to a PhosphorImager.

UL42-DNA Cross-linking—The thiol-tethered oligonucleotide (5’-taccgcagccatcagagt-3’) was synthesized using the method previously described (34). The thiol tether was attached to the backbone phosphate between bases 11 and 12. The unmodified oligonucleotide and its complementary strand were purchased polyacrylamide gel-purified from Integrated DNA Technologies, Inc. Double-stranded DNA was formed by mixing the two complementary oligonucleotides 1:1 in a buffer containing 25 mM NaCl and 15 mM Tris-HCl (pH 7.5). The mixture was heated to 85 °C and then cooled slowly to room temperature. Cross-linking reactions were performed by mixing either wild-type or mutant UL42 proteins (1 μM) with either unmodified or thiol-tethered double-stranded DNA (2 μM) in 15 μl of reaction buffer (30 mM Tris-HCl (pH 7.5), 30 mM NaCl, and 10 μM dithiothreitol (DTT)) for 1 h at room temperature in the presence or absence of 2 mM DTT. The reactions were stopped by capping the free thiol groups with S-methyl methane thiosulfate (40 mM). The quenched reaction mixtures were analyzed on a 10% SDS-polyacrylamide gel under nonreducing conditions. The gel was stained using SimplyBlue™ Safe Stain (Invitrogen) overnight and destained in water.

RESULTS

Lack of Effect of UL30 C Terminus on UL42 Binding to DNA—We wished to investigate whether the presence of the UL30 C terminus, which interacts with UL42 (13, 21, 27), alters the affinity of UL42 for DNA, as previously seen for HCMV UL44 and the C terminus of its catalytic subunit, UL54 (26). To address this question we performed filter binding assays in the presence or absence of an excess of a 36-residue peptide that corresponds to the C terminus of UL30 and is sufficient to bind to UL42 (21, 27). We added increasing amounts of protein to 1 fmol of 5’-end labeled 102-bp DNA and measured the fraction of bound DNA, which was plotted against protein concentration (Fig. 1). The plots were very similar in the presence or absence of the UL30 peptide, providing no evidence for a change in affinity of UL42 for DNA upon binding of the UL30 C terminus. We, therefore, conducted all subsequent assays in the absence of this peptide.

Effects of UL42 Back Face Arginine to Lysine Substitutions on Binding to DNA—The crystal structure of UL42 reveals a positively charged back face (face opposite to that which binds UL30) with four arginines (Arg-113, Arg-182, Arg-279, and Arg-280) that are conserved among alphaherpesviral homologues of UL42 (Fig. 2). A previous study by Randell et al. (23) had shown that substitution of any of these four arginines with alanines results in decreased affinity of UL42 for DNA with an even greater reduction in DNA binding observed when all four of the arginines were substituted with alanines. To test whether the positive charge of the arginines was the feature important

![Graph showing the lack of effect of UL30 peptide on UL42 binding to DNA.](http://www.jbc.org/)

**FIGURE 1.** Lack of effect of UL30 peptide on UL42 binding to DNA. Varying amounts of UL42 were incubated with radiolabeled DNA in the presence (open triangles) or absence (closed triangles) of a 36-residue peptide corresponding to the C terminus of UL30, and the fraction of DNA bound was measured using a filter binding assay. Error bars represent S.E. from three experiments. wt, wild type.

![Diagram of residues substituted on the basic back face of UL42.](http://www.jbc.org/)

**FIGURE 2.** Residues substituted on the basic back face of UL42. The peptide backbone of the UL42 structure (21) (lacking the UL30 peptide) is shown in gray. The side chains of conserved arginine residues, Arg-113, Arg-182, Arg-279, and Arg-280 and that of the conserved glutamine residue Gln-282 are shown in black.
for DNA binding, we substituted each of the arginine residues with lysines, individually or together. We then measured the affinities of the mutant proteins for double-stranded DNA using a filter binding assay. Because the assay was performed under conditions where the DNA concentration was very low, we could use saturation isotherm analysis to calculate the apparent dissociation constant ($K_d$) from the concentration of the protein that led to half saturation. The $K_d$ values are apparent rather than absolute because for any length of DNA longer than the binding site for UL42, there are multiple potential binding sites. Most single mutants exhibited apparent $K_d$ values for DNA similar to that of the wild-type UL42 protein (Fig. 3, Table 1). The R113K mutant showed an ~3-fold increase in apparent $K_d$ for DNA compared with the wild-type protein. However, this increase was small in comparison to the more than 25-fold increase in apparent $K_d$ of the R113A mutant, measured in parallel using a filter binding assay (Fig. 3, Table 1), and the 22-fold increase for the R113A mutant found previously using an electrophoretic mobility shift assay (23). The mutant that had all four arginines substituted with lysines also showed little or no decrease in affinity for DNA when compared with wild-type UL42 (Fig. 3, Table 1). All of these mutants retained wild-type affinity for the C-terminal 36 residues of polymerase as measured by isothermal titration calorimetry, indicating that they were properly folded (Table 1). These results combined with those from Randell et al. (23) suggest that it is the positive charge of the arginines on the back face of UL42 that is important for DNA binding.

**A UL42 Mutant That Binds DNA with Higher Affinity**—To investigate further the role of the back face of UL42 in DNA binding, we asked whether the affinity of UL42 for DNA could be increased by increasing the amount of positive charge on this surface.UL42 contains a glutamine residue on the back face that is conserved among alphaherpesvirus homologues (Fig. 2). We engineered a mutant of UL42 in which this glutamine was substituted with an arginine residue (Q282R). When compared side-by-side with wild-type UL42 in the filter binding assay (Fig. 4A), this mutant exhibited an ~4-fold increase in affinity for DNA (apparent $K_d$ for mutant, 0.68 ± 0.1 nM; apparent $K_d$ for wild type, 2.4 ± 0.4 nM). This mutant retained wild-type affinity for the C-terminal 36 residues of polymerase as measured using an FP assay (Fig. 4B), indicating that the Q282R mutation has a specific effect on DNA binding. These data further support our hypothesis that the back face of UL42 mediates DNA binding.

**The Substitutions Exert Modest Effects at Most on Long Chain DNA Synthesis by UL30/UL42**—We have previously observed that single arginine to alanine substitutions on the back face of UL42 reduced long chain DNA synthesis by the UL30/UL42 complex (23). We tested each of the substitution mutants constructed here for their ability to stimulate long chain DNA synthesis by UL30, which is a measure of holoenzyme processivity. The assay used measures the incorporation of radiolabeled nucleotides on an oligo(dT)-primed poly(dA) template and analysis on alkaline-agarose gels. As previously observed (e.g. Ref. 23) in the absence of UL42, only short products were faintly detected (Fig. 5), and no products could be observed using wild-type UL42 alone. All of the UL42 arginine to lysine substitution mutants stimulated long chain DNA synthesis, although two of the single substitutions (R113K and

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**TABLE 1**

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Apparent $K_d$ for DNA</th>
<th>$K_d$ for peptide A</th>
</tr>
</thead>
<tbody>
<tr>
<td>None (wild type)</td>
<td>4.3 ± 0.6</td>
<td>2.5 ± 0.4</td>
</tr>
<tr>
<td>R113K</td>
<td>14 ± 2</td>
<td>1.1 ± 1.1</td>
</tr>
<tr>
<td>R182K</td>
<td>2.7 ± 0.3</td>
<td>2.5 ± 0.9</td>
</tr>
<tr>
<td>R279K</td>
<td>4.6 ± 0.6</td>
<td>2.9 ± 0.8</td>
</tr>
<tr>
<td>R280K</td>
<td>3.4 ± 0.6</td>
<td>2.3 ± 0.6</td>
</tr>
<tr>
<td>R113K/R182K/R279K/R280K</td>
<td>4.8 ± 0.5</td>
<td>1.7 ± 0.5</td>
</tr>
<tr>
<td>R113A</td>
<td>&gt;100</td>
<td>1.7 ± 0.03*</td>
</tr>
</tbody>
</table>

* Previously published (23).
**HSV UL42 Basic Face Mediates DNA Binding**

![Figure 5](http://www.jbc.org/content/10.1074/jbc.M804100200)

**FIGURE 5. Arginine to lysine and glutamine to arginine substitutions at most modestly affect long chain DNA synthesis by UL30/UL42.** Long chain DNA synthesis was measured by the incorporation of radiolabeled dTTP on a poly(dA)/oligo(dT) primer-template followed by separation of products on a 4% alkaline agarose gel. Products were visualized with a PhosphorImager. Lane 1 contained products synthesized by 200 fmol of UL30 in the absence of UL42, whereas lane 2 contained the products of a reaction containing 800 fmol of wild-type (wt) UL42. Reaction mixtures analyzed in the remaining odd-numbered lanes contained 200 fmol of UL30 and 800 fmol of the UL42 (wild type or mutant) indicated above the phosphorimage, and reaction mixtures analyzed in the remaining even-numbered lanes contained 200 fmol of polymerase (Pol) and 1200 fmol of the indicated UL42. The positions of long and short products are indicated to the left of the phosphorimage. Similar results were obtained in two other experiments.

R280K) and the quadruple substitution resulted in somewhat decreased incorporation and slightly shorter products (Fig. 5). The Q282R substitution that results in higher affinity binding to DNA also resulted in slightly less incorporation and shorter products. In contrast, an arginine to alanine substitution mutant (R280A) was much more defective for stimulation of long chain DNA synthesis in these assays, resulting in much less incorporation and only short products. Thus, the lysine and Q282R substitutions, as expected from their modest effects on DNA binding, exerted, at most, modest effects on long chain DNA synthesis.

**Disulfide Cross-linking of UL42 to a Thiol-modified DNA—** Our mutational analysis indicated that positive charge on the back face of UL42 is important for DNA binding, but it did not necessarily show that this surface of UL42 directly contacts DNA. We, therefore, used a disulfide cross-linking strategy to covalently trap UL42 bound to DNA. We synthesized an 18-bp DNA molecule that has a single thiol tether (34) attached to a backbone phosphate (Fig. 6A). We based the location of this tether on our hypothesis that the positive charge of the back face interacts with the negatively charged phosphate backbone of DNA (indeed, efforts to cross-link UL42 to DNA via base moieties on the DNA have failed). Wild-type UL42 has four cysteines (Cys-31, Cys-218, Cys-272, Cys-300; Fig. 5B) and 6), two of which (Cys-218 and Cys-300) are exposed on the surface of the protein in the crystal structure of UL42 (21). At least some of these cysteines are accessible in solution, as wild-type UL42 can be modified by reagents that react with cysteines. However, none of these cysteines is on the back face of UL42. We, therefore, chose to incorporate a cysteine residue onto this surface by engineering two mutants, each with a substitution of a cysteine for an arginine residue on the back face. One of these arginines was Arg-182 as the R182A mutant exhibited the smallest decrease in affinity for DNA as compared with the wild-type protein in our previous study (23). The other arginine was Arg-51, which has a favorable location on the basic face of UL42 (Fig. 6B). As a control we measured the affinities of the R182C and R51C UL42 proteins for the C-terminal UL30 peptide by isothermal titration calorimetry. Both mutant proteins had $K_v$ values for this peptide (R51C, 2.2 $\mu$M; R182C, 3.5 $\mu$M) similar to that of wild type (2.5 $\mu$M), indicating that they were properly folded.

We then tested wild-type and mutant UL42 proteins for disulfide cross-linking with the thiol-tethered DNA. Wild-type UL42 did not detectably cross-link this DNA (Fig. 6C) as predicted from the lack of cysteines on its back face. Nor did it cross-link unmodified DNA. However, upon substituting either Arg-182 or Arg-51 (Fig. 6B) with a cysteine residue, we were able to cross-link UL42 to this DNA, as can be seen from the appearance of a new band on an SDS-polyacrylamide gel whose molecular weight corresponds to UL42 + 18 bp DNA (Fig. 6C). We did not observe any cross-linking in the presence of 2 mM dithiothreitol or when control DNA that does not contain a thiol tether was used in the reaction (Fig. 6, C and D), showing that cross-linking occurred through a disulfide linkage and was specific to the presence of the thiol tether on DNA. Thus, cross-linking required engineering of the cysteine residues onto the basic face of UL42, which suggests that this face contacts DNA.

**Ion Release during Binding of UL42 to DNA—** The results of the above experiments suggested that positively charged residues on the back face of UL42 bind to DNA via charge-charge interactions with the negatively charged phosphate backbone of DNA. Previous studies of protein-DNA binding involving charge-charge interactions have shown that affinity decreases with increasing salt concentration, and binding is accompanied by the release of bound ions. One can estimate the number of ions released during binding by analyzing the dependence of the observed equilibrium binding constant ($K_{obs}$) on ionic strength. In particular, according to the binding theory of Record et al. (31, 32), the log of $K_{obs}$ is a linear function of the log of monovalent ion concentration ($M^+$), and from the slope of such a graph the number of phosphates neutralized and, thus, ions released, $Z$, can be calculated from the equation $d\log K_{obs}/d\log[M^+] = -Z\psi$, where $\psi$ is a constant (0.88 for duplex B-form DNA). If the ions released are cations, then the number released provides an estimate of the number of charge-charge interactions between the protein and the DNA. Criteria for determining whether the ions released are cations include finding similar numbers of ions released when salts composed of the same cation but different anions are used and finding roughly half the number of ions released when comparing divalent to monovalent cations.

We, therefore, used filter binding assays to measure the dependence of $K_{obs}$ of the binding of UL42 to a 30-bp DNA interaction in the presence of varying concentrations of NaCl (Fig. 7A), NaCH$_3$CO$_2$ (Fig. 7B), and MgCl$_2$ (Fig. 7C) in buffers in which the only other salt was 1 mM Tris-HCl (a concentra-

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4 J. Randell and D. M. Coen, unpublished observations.

5 G. Komazin-Meredith and D. M. Coen, unpublished observations.
tion much lower than those of the varied salts). In all cases affinity decreased with increasing salt concentration, and the log $K_{\text{obs}}$ was a linear function of log cation concentration. When the values for the two monovalent salts (NaCl and NaCH$_3$CO$_2$) were plotted and the data fitted by least squares analysis, the slopes were $-1.6 \pm 0.2$ and $-2.6 \pm 0.2$, respectively. Dividing these slopes by the constant $\psi$ (0.88) yields values corresponding to 1.8 and 3.0 phosphates neutralized, respectively. This suggested that $\sim$2–3 monovalent ions are released per binding event. The slopes were fairly similar despite the different anions used, and the $K_{\text{obs}}$ values obtained in the different salts at similar ionic strength were also fairly similar, ranging from being 3-fold different at 25 mM to being nearly identical at 75 and 100 mM. These small differences in $K_{\text{obs}}$ contrast with the 40-fold difference observed in a similar study of the non-sequence-specific interaction of E. coli lac repressor with DNA (30) and suggest that the contribution of anion release was small. When the concentration of MgCl$_2$ was varied (Fig. 7C), the least squares analysis yielded a slope of $-1.1 \pm 0.1$. Dividing by the relevant constant $\psi$ (0.47) yields a value corresponding to 2.3 phosphates neutralized per UL42-DNA binding event, which is in line with the estimate of 2–3 monovalent cations released. However, the slope obtained in MgCl$_2$ was $>0.53$ of that of the slope in NaCl (see “Experimental Procedures” for the relevant equation), raising the possibility of some contribution of anion release during binding. The numbers and kinds of ions released are discussed below in relationship to our other data. Regardless, the results show that UL42 binding to DNA is accompanied by ion release.

**DISCUSSION**

In this study we found that a peptide corresponding to the C terminus of UL30 does not affect UL42 binding to DNA. We then found that histidine residues on the basic back face of UL42, whose substitution with alanine decreases DNA binding, can be substituted with lysine without affecting DNA binding, whereas substitution of a glutamine on this surface with arginine increases DNA binding. Incorporation of cysteine residues onto this surface permitted disulfide cross-linking to thiol-tethered DNA, whereas unsubstituted UL42 did not covalent this DNA. All of these results taken together strongly suggest that the basic back face of UL42 contacts DNA and are consistent with arginine residues on this surface of DNA interacting with the negatively charged phosphate backbone of DNA. Consistent with this idea, measurements of binding in
different concentrations of various salts provided evidence for
ion release upon binding of UL42 to DNA, although the num-
ber of charge-charge interactions estimated from this analysis
was relatively small. Below we discuss these results and relate
them to hypotheses regarding how UL42 binds DNA and medi-
ates processive DNA synthesis.

Lack of Effect of UL30 C Terminus on UL42 Binding to DNA—
We were motivated to test the effect of the C terminus of
UL30 on the interaction of UL42 with DNA because the C ter-
mminus of HCMV UL54 (the catalytic subunit of HCMV DNA
polymerase) increases the affinity of HCMV UL44 for DNA
(26). Because the crystal structure of UL44 bound to the UL54 C
terminus is in a different conformation than that of unliganded
UL54 (26), it is possible that the increase in affinity is due to this
conformational change. However, we failed to observe any
change in the affinity of UL42 for DNA in the presence of the
UL30 C terminus. The results then lend no support to the pro-
posal that interaction of UL42 with UL30 leads to a conforma-
tional change that results in increased affinity of the polymerase
for DNA (25). Nevertheless, we cannot rule out that binding of the
UL30 C terminus does alter the conformation of UL42 in some manner. A crystal structure of unliganded UL42 could
help address this issue.

The lack of effect of the C terminus of the catalytic subunit on
DNA binding provides yet another example of differences
between HSV UL42 and HCMV UL44 despite their similar
structural folds, high affinity DNA binding, and basic back
faces. These differences include differences in quaternary
structure, the nature of the interaction with their cognate cat-
alytic subunits, composition of the basic back face, affinity for
single-stranded DNA, and the number of charge-charge inter-
actions estimated upon binding to DNA (this study and Refs.
21, 22, 26, 27, 29, 35, and 36).

Positive Charge on the Back Face of UL42 Is Important for
DNA Binding—As anticipated, substitutions of arginine resi-
dues on the back face of UL42 with lysines had substantially less
effect on DNA binding than substitutions with alanine. This
strongly suggests that it is the basic nature of the arginine resi-
dues that is important for DNA binding. On the other hand, we
wondered whether the substitutions with lysines, whose ε
amino groups are thought to form weaker interactions with
DNA than the guanidino groups of arginines, would result in
reduced affinity for DNA. Indeed, previously we have specu-
lated that HCMV UL44, whose back face is lysine-rich, might
contain lysines rather than arginines to compensate for the
larger surface area formed by being a homodimer, so that it
would bind DNA with an affinity similar to that of HSV UL42
(22). We had reasoned that tight binding to DNA might slow
diffusion of these viral processivity factors and, thus, slow po-
lymerase elongation. However, the UL42 mutant with lysines
replacing all four arginines did not bind DNA with less affinity
than did wild type. Oddly, replacing one of the arginines (Arg-
113) with lysine did result in a small decrease in affinity, and this
substitution, one other single substitution (R280K), and the
quadruple substitution resulted in modest decreases in long
chain DNA synthesis by UL30/UL42. Thus, there may be subtle
effects of these changes on the UL42-DNA interaction. Never-
theless, none of these changes affect UL42 function nearly as
much as arginine to alanine substitutions.

Our finding that a mutant in which glutamine 282 was sub-
stituted with arginine binds DNA more tightly provides further
evidence that the back face of UL42 interacts with DNA. This
finding also raises the question of why this residue, which is one
member of a trail of residues conserved among alphaherpesvi-
ruses (23), is not ordinarily positively charged. Again, one pos-
sibility is that too much positive charge might result in such
tight binding to DNA that diffusion of UL42 on DNA might
slow and, thus, might “brake” the elongation of HSV DNA po-
lymerase. This conceivably could account for the slightly
reduced long chain DNA synthesis observed with this mutant.
It will be interesting to test whether the Q282R substitution
does indeed slow diffusion or reduce HSV DNA replication.

Why Are More Positively Charged Residues Important for
Binding Than the Estimated Number of Charge-Charge
Interactions?—The results of our studies of DNA binding by
UL42 in varying concentrations of salts qualitatively support a
model of binding mediated by electrostatic interactions
between positively charged residues of UL42 and negatively
charged phosphates on the DNA in that affinity decreased as
ionic strength increased. Moreover, the analysis indicated that
binding was accompanied by cation release. However, these
results provided a maximum estimate of ~3 cations released,
implying a maximum of ~3 charge-charge interactions per
binding event. This value is maximal both because it is the larg-
est one obtained experimentally among the three different salts
tested and also because of the possibility of anion release upon
binding, which could not be excluded. However, any such anion
effects would most likely be due to the release of anions that,
in the absence of DNA, bind to positively charged residues of
UL42 and would, thus, be consistent with these residues being
important for DNA binding.

Regardless, the number of charge-charge interactions esti-
mated is less than the four conserved arginine residues that
mutational analysis has identified as being important for DNA
binding (23). Moreover, the back face of UL42 includes several
less-well conserved arginines and lysines (Arg-51, Lys-105,
Arg-106, Lys-187, Arg-275) whose positive charge might also
contribute to DNA binding. Interestingly, we have also
detected fewer charge-charge interactions between HCMV
UL44 and DNA than the number of basic residues that have been
implicated as being important for binding (29). We offer
two possible explanations for the apparent discrepancy
between the number of charge-charge interactions detected
and the number of basic residues implicated as being important
in binding. One possibility is that each important basic residue
contributes only partially to charge-charge interactions with
negatively charged phosphates. A second possible explanation
is that, as UL42 diffuses on DNA, only three or fewer basic
residues interact with DNA on average at any time, which
might, in fact, facilitate diffusion. Indeed, it is likely that there
is no single arrangement of protein-DNA contacts during diffu-
sion. Some combination of these mechanisms is also possible.

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Relationship of Electrostatic Binding to Processivity Factor Function—Most proteins that bind specific sequences on DNA make direct contacts with the base pairs in the grooves of DNA. Electrostatic interactions between basic amino acids and the negatively charged DNA backbone can also contribute to sequence specific DNA binding, and most proteins that interact with specific sites on DNA are also able to bind nonspecifically to DNA but with reduced affinity. One example of a protein that is able to bind both specific and nonspecific DNA as well as diffuse on nonspecific DNA is the lac repressor. The structure of lac repressor has been solved both in complex with specific and nonspecific DNA (37, 38). Although in the specific complex lac repressor forms direct interactions with the base pairs of the cognate operator sequence, in the nonspecific complex it forms almost exclusively electrostatic interactions (37, 38). This nonspecific binding is thought to accelerate the search for target sequences by scanning long stretches of DNA via sliding (37–39).

This mode of diffusion on DNA can be contrasted with that of PCNA and other sliding clamp processivity factors, which are generally acidic except for the interior of their rings, which is basic (1, 3, 5, 40). Studies of alanine substitution mutants suggest that these basic residues are important for loading of PCNA onto DNA rather than for long chain DNA synthesis (41). This contrasts with UL42 arginine to alanine substitution mutants, which exhibit reduced long chain DNA synthesis (23). It is thought that after sliding clamps are loaded on DNA by accessory proteins, they encircle DNA as rings with a central channel large enough to allow DNA to slide through without tight contacts with the protein (6).

Thus, although HSV UL42 shares a protein fold and processivity factor function with PCNA and the sliding clamps, it appears to interact with DNA using charge-charge interactions in a manner akin to lac repressor when it binds to and diffuses on DNAs for which it lacks sequence specificity. What is less clear is how UL42 binds DNA as tightly as it does with fewer charge-charge interactions (comparing this study with those on lac repressor, e.g. Ref. 30) yet is still able to diffuse on DNA. A clearer picture of how UL42 and other processivity factors interact with DNA would greatly benefit from high resolution structures of these proteins in complex with DNA.

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The Positively Charged Surface of Herpes Simplex Virus UL42 Mediates DNA Binding

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