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Interaction with Nucleic Acids and Stimulation of the Viral DNA Polymerase by the Herpes Simplex Virus Type 1 Major DNA-Binding Protein

WILLIAM T. RUYECHAN* AND ANNA C. WEIR

Department of Biochemistry, Uniformed Services University of the Health Sciences, Bethesda, Maryland 20814-4799

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The interaction of the herpes simplex virus type 1 (HSV-1) major DNA-binding protein, infected-cell polypeptide 8 (ICP8), with nucleic acids has been examined by a filter-binding assay and electron microscopy. Filter-binding assays done over a broad pH range indicated that the optimum pH for the protein-DNA interaction is ~7.6. Heat inactivation studies showed that ICP8 is stable at temperatures up to 40°C with a rapid loss of binding activity on incubation at 45°C and above. Competition binding experiments have established the following relative affinities of ICP8 for the following nucleic acids: single-stranded HSV-1 DNA ≃ bacteriophage fd DNA > polyriboadenylate ≫ double-stranded HSV-1 DNA ≃ d(pCpT)$_5$. Observation of negatively stained ICP8–single-stranded DNA complexes indicated that ICP8 binds along the length of the DNA in a regular repeating fashion. The average width of these complexes is 9.3 ± 0.8 nms. Finally, addition of purified ICP8 to HSV-1 DNA polymerase reactions resulted in a stimulation of the viral polymerase activity.

The major DNA-binding protein found in cells infected with herpes simplex virus type 1 (HSV-1) is a member of the β or "early" temporal class of virus-specific polypeptides. The β polypeptides also include the viral DNA polymerase and thymidine kinase, and their peak synthesis coincides with the peak synthesis of viral DNA (8, 15). The molecular weight of the HSV-1 DNA-binding protein is 125,000 to 128,000, as determined by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis, and this protein has been designated infected-cell polypeptide 8 (ICP8) based on its electrophoretic mobility relative to other infected-cell proteins (16, 22).

ICP8 binds more tightly to single- than to double-stranded DNA based on DNA cellulose chromatography of infected-cell extracts (1, 9, 18, 20). Purified ICP8 is capable of melting polydeoxyadenylate-polydeoxythymidylate under conditions in which the polynucleotides form stable duplexes (17). Recent work has shown that the ICP8–single-stranded DNA interaction is cooperative (22). The stoichiometry of binding at saturation is approximately 40 nucleotides per protein monomer, which translates to a protein/DNA weight ratio of 10:1. Measurements of single-stranded DNA-protein complexes indicate that the DNA is held in a configuration with an apparent base spacing of ~0.13 nm per base.

There is substantial evidence that ICP8 is essential for viral DNA replication. Antibody against ICP8 inhibits DNA synthesis in chromatin isolated from HSV-infected cells (17). Temperature-sensitive mutants mapping within the coding sequences for ICP8 have DNA-negative phenotypes, and several of these mutants encode proteins which exhibit temperature-sensitive DNA-binding activities (3, 12, 13, 29). A recent report indicates that ICP8 is also involved in the regulation of viral gene expression and that this role is independent of the requirement of ICP8 for viral DNA replication (5). Finally, ICP8 may be a group-specific antigen since antibodies against ICP8 cross-react with analogous proteins coded by other herpesviruses (14). In the work reported here we have examined several aspects of the interaction of ICP8 with polynucleotides and the effect of ICP8 on the activity of HSV-1 DNA polymerase.

* Corresponding author.

MATERIALS AND METHODS

Cells and viruses. HSV-1 strain mP (7) was propagated in Vero cell monolayers by standard procedures (16). Initial stocks of the virus were provided by David Knipe.

Enzymes and reagents. Pancreatic DNase and RNase and salmon sperm DNA were purchased from Sigma Chemical Co. (St. Louis, Mo.). DEAE-cellulose powder was purchased from Bio-Rad Laboratories (Richmond, Calif.), and phosphocellulose powder (P11) was purchased from Whatman, Inc. (Clifton, N.J.). φX174 viral DNA and single-stranded DNA agarose were purchased from Bethesda Research Laboratories, Inc. (Bethesda, Md.). d(pCpT)$_5$ was purchased from Collaborative Research (Waltham, Mass.). HSV-1 DNA was isolated from cells infected with strain mP, using the sodium iodide procedure of Walboomers and ter Schegget (27). Bacteriophage fd DNA, exonuclease III-treated calf thymus DNA, and T4 gene 32 protein were the gift of Lucy M. S. Chang. DNA polymerase α isolated from calf thymus was the gift of Andrew Holmes. [^{3}H]dTTP (60 Ci/mmol) was purchased from ICN Pharmaceuticals Inc. (Irvine, Calif.). dTTP, dATP, dCTP, and dGTP were provided by J. Hay.

Purification of ICP8 and HSV-1 DNA polymerase. ICP8 was routinely prepared from 25 confluent 2.5-liter roller bottles of Vero cells infected with HSV-1 strain mP at an input multiplicity of 20 PFU per cell. Extraction of the cells and purification through chromatography on DEAE-cellulose and phosphocellulose were carried out as previously described (17, 22). Chromatography was carried out with a Frac 100 chromatography system (Pharmacia Fine Chemicals Inc., Piscataway, N.J.) which included a UV flow cell monitor, an automated fraction collector, and a stripchart recorder. ICP8-containing fractions were adjusted to 500 μg/ml in bovine serum albumin (BSA), dialyzed against low-salt buffer (50 mM KCl, 20 mM Tris-hydrochloride [pH 7.5], 0.5 mM dithiothreitol, 50% glycerol), and applied to a 20-ml single-stranded DNA agarose column which had been

prewashed with BSA. The column was eluted with a linear 0.05 to 2.0 M KCl gradient, and fractions containing pure ICP8 as determined by SDS gel electrophoresis were obtained at KCl concentrations of 1.2 to 1.5 M. These fractions were dialyzed against TEK buffer (150 mM KCl, 10 mM Tris-hydrochloride [pH 7.6], 1 mM EDTA, 0.5 mM dithiothreitol, 20% glycerol) and stored at 4°C until use. The average yield of protein was 50 to 100 μg. Protein was quantitated with a Bio-Rad protein assay kit.

HSV-1 DNA polymerase was copurified with ICP8 from a 40-roller bottle preparation of infected cells, using the method of Powell and Purifoy (19). The extraction procedures and two initial chromatography steps are identical to those described for ICP8. HSV-1 polymerase activity eluting from the columns was assayed as described below. The presence of ICP8 was detected by gel electrophoresis. ICP8 and HSV-1 DNA polymerase activity were cleanly separated in the phosphocellulose chromatography step. Appropriate fractions were then dialyzed against low-salt buffer and applied to separate single-stranded DNA-agarose columns. Purification of ICP8 proceeded exactly as described above. The DNA-agarose column to which the DNA polymerase-containing fractions had been adsorbed was washed with low-salt buffer and eluted with a linear 0.05 to 1.0 M KCl gradient. Fractions containing polymerase activity were obtained at between 0.5 and 0.7 M KCl. The fractions were made 50% in glycerol and stored at −20°C.

DNA polymerase assays. The polymerase assay used in these studies is similar to that used by Powell and Purifoy (19). The reaction mixture contained 100 mM KCl, 8.0 mM $MgCl_2$, 2.0 mM 2-mercaptoethanol, 20.0 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) buffer (pH 7.8), 12.5 or 50 μg of heat-denatured salmon sperm DNA, 0.26 mM each dGTP, dATP, and dCTP, 0.06 mM dTTP, 1.5 μCi of [^{3}H]dTTP (60 Ci/mmol), and 10 to 20 μl of a given HSV-1 DNA polymerase fraction. The final sample volume was 100 μl. The reaction mixtures were incubated at 37°C for 30 min. After incubation the samples were spotted onto squares of Whatman no. 2 paper and washed with 5% trichloroacetic acid–10 mM sodium pyrophosphate at 0°C. The squares were then washed sequentially with 5% trichloroacetic acid and 95% ethanol, dried, and counted. The efficiency of counting for tritium was ~30%. One unit of HSV-1 DNA polymerase activity was defined as the amount of enzyme catalyzing the incorporation of 0.1 nmol of dTMP per h under the standard assay conditions. DNA polymerase α activity was assayed at 50 mM KCl. All other reaction conditions were identical to those used with the HSV-1 polymerase.

Filter-binding assay. The filter-binding assay for detection of ICP8-DNA interactions has been described in a previous report from this laboratory (22). Sonicated HSV-1 DNA was labeled to a specific activity of 3.6×10^5 dpm/μg with [^{3}H]dCTP (50 Ci/mmol), using a nick translation kit (Amersham Corp., Arlington Heights, Ill.). The DNA was then dialyzed against 0.01 M Tris-hydrochloride (pH 7.6)–0.001 M EDTA and denatured before use by heating to 100°C for 5 min. The standard assay mix contained 0.02 to 0.05 μg of labeled DNA and 0.2 to 0.5 μg of ICP8. Other components were added as described below. Unless otherwise indicated all assays were carried out at pH 7.6 and with 0.15 M KCl in a volume of 100 μl. The mixtures were incubated for 10 min at 20°C. After incubation 80-μl aliquots were adsorbed to nitrocellulose filters on a Hoefer multichannel filtration manifold. Filtration speed, washing, and counting of the filters were exactly as described previously.

Electron microscopy of protein-DNA complexes. Negatively stained ICP8–single-stranded DNA complexes were prepared by mixing 0.1 to 0.2 μg of φX174 viral DNA with 1.0 to 2.0 μg of ICP8 or T4 gene 32 protein in TEK buffer in a final volume of 100 μl. After an initial 10-min incubation at 20°C, 1.25 μl of 8% glutaraldehyde was added and the sample was incubated for an additional 10 min (22, 23). A drop of the sample was adsorbed to airglow-treated 200-mesh carbon-coated grids for 60 s. The excess sample was blotted away, and the grids were stained with 2% phosphotungstic acid (pH 7.4) for 60 s, blotted, and air dried. The resulting complexes were observed with a Zeiss EM 10A transmission electron microscope and photographed at a magnification of ×80,000.

RESULTS

Purification of ICP8 and HSV-1 DNA polymerase. ICP8 and HSV-1 DNA polymerase were purified as described above. The presence of ICP8 was monitored by SDS gel electrophoresis. HSV-1 DNA polymerase activity was assayed under high-salt (100 mM KCl) conditions specific for the viral enzyme. Purification of both proteins from the same extract was straightforward. ICP8 and the viral DNA polymerase activity partially overlapped on elution from DEAE-cellulose. Relevant fractions were pooled, dialyzed, and adsorbed to a 30-ml phosphocellulose column. Elution of this column with a 0.05 to 0.40 M KCl gradient resulted in a clean separation of ICP8 and the DNA polymerase activity. Further purification of the individual proteins was then carried out by DNA-agarose chromatography as described above. The elution profile of the ICP8 copurified with the polymerase was identical to that obtained from the standard ICP8 preparations.

SDS gel electrophoresis of peak ICP8 fractions showed a single protein band with a molecular weight of ~125,000 (Fig. 1, lane A). ICP8 activity in these fractions was monitored by filter binding and electron microscopy of DNA-protein complexes. SDS gels of the peak polymerase frac-

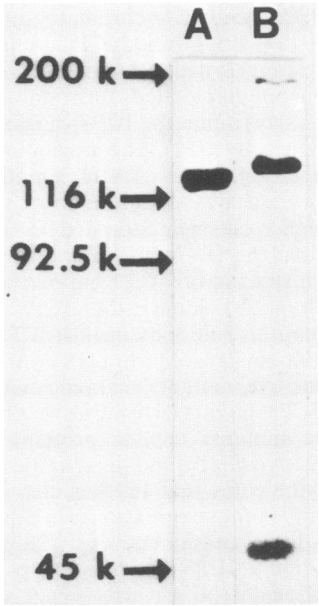

FIG. 1. A 9% SDS-polyacrylamide gel of peak ICP8 (A) and HSV-1 DNA polymerase (B) fractions eluted from separate single-stranded DNA agarose columns. Proteins were visualized by means of a silver-staining procedure (30).

tions showed a major protein band with a molecular weight of ~148,000 (Fig. 1, lane B). A protein of similar molecular weight was identified as the HSV DNA polymerase by Powell and Purifoy (19). Two contaminating proteins with molecular weights of 185,000 and 51,000 were also present. The lower-molecular-weight protein may correspond to the 54,000 contaminant seen by Powell and Purifoy (19). The specific activity of the peak DNA polymerase fraction was 7,200 U/mg of total protein.

pH dependence and thermolability of ICP8. The pH dependence of the ICP8–single-stranded DNA interaction and the thermolability of the DNA-binding activity of ICP8 were examined by using a filter-binding assay. For the pH-dependence studies aliquots of ICP8 were dialyzed against 0.01 M acetate buffer (pH 5.5), 0.01 M phosphate buffer (pH 6.5), and 0.01 M Tris-hydrochloride buffers (pH 8.5 and 9.5). All of the buffers contained 150 mM KCl, 1 mM EDTA, 0.1 mM dithiothreitol, and 20% glycerol. Standard filter-binding assays were performed in duplicate at each of the above pH values as well as at pH 7.6. The results presented in Fig. 2 indicate that the single-stranded DNA-binding activity of ICP8 is maximal near neutrality and is quite sensitive to both slightly acidic and slightly alkaline pH. The sensitivity to slightly alkaline pH is particularly marked as a pH of 8.5 reduced the observed binding by >90%.

The thermolability of ICP8 was tested by incubating aliquots of ICP8 at 4, 25, 37, 40, 45, 50, 75, and 100°C for 10 min. The aliquots were ice quenched and allowed to return to 25°C. Standard filter-binding assays were performed in triplicate with protein from each aliquot. The results shown in Fig. 3 indicate that the DNA-binding activity is stable, within experimental error, through incubations at 40°C, although the scatter in the 40°C point suggests some loss of activity. Protein aliquots incubated at 50°C and above displayed no observable binding. To determine if the presence of exogenous protein could protect ICP8 from thermal inactivation, a second set of ICP8 aliquots was adjusted to 50 μg of BSA per ml and subjected to the same incubations described above. Assays performed in triplicate with the BSA-containing samples gave an inactivation curve identical to that observed with ICP8 alone. Control assays done with BSA alone showed that the presence of BSA had no effect on the retention of single-stranded DNA on the filters.

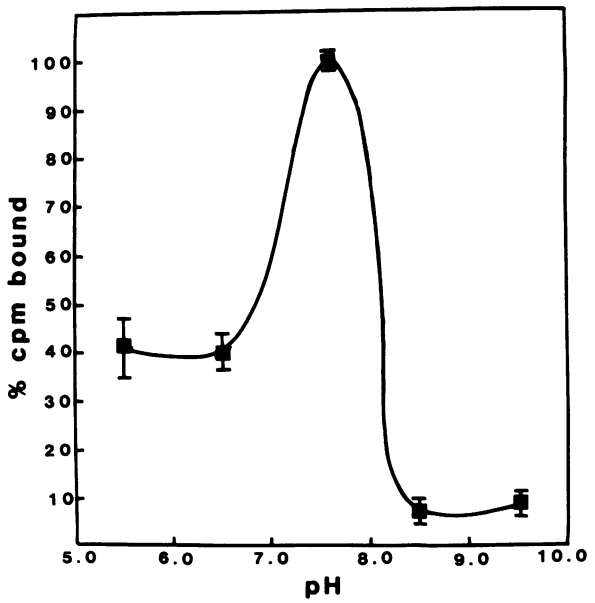

FIG. 2. pH dependence of the ICP8–single-stranded DNA interaction. Points represent the average of duplicate filter-binding assays carried out at the indicated pH values.

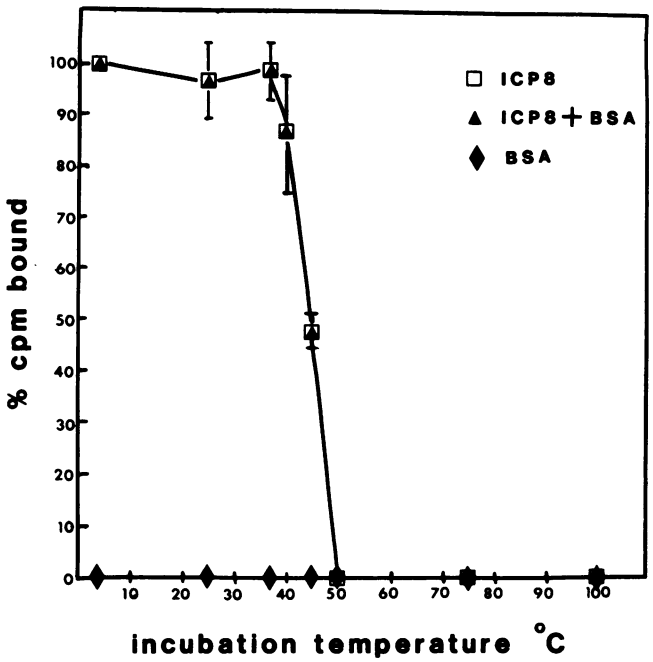

FIG. 3. Thermal inactivation of purified ICP8. Points represent the average of triplicate filter-binding assays carried out with aliquots of ICP8 which had been incubated at the indicated temperatures in the presence and absence of BSA. BSA alone showed no DNA-binding activity.

Filter binding of single-stranded HSV-1 DNA-ICP8 complexes in the presence of competing nucleic acids. The relative affinity of ICP8 for a variety of nucleic acids was examined by using a competitive filter-binding assay. A 0.05-μg portion of ^{3}H-labeled single-stranded HSV-1 DNA was mixed with 0.05, 0.10, 0.15, and 0.20 μg of the following unlabeled nucleic acids: single-stranded HSV-1 DNA, double-stranded HSV-1 DNA, phage fd DNA, polyriboadenylate [poly(rA)], and $d(pCpT)_5$. A 0.6-μg amount of ICP8 was then added and filter-binding assays were carried out in duplicate under standard conditions. The results are presented in Fig. 4. Single-stranded HSV-1 DNA appeared to compete slightly more efficiently than did fd DNA, although the two were equivalent within experimental error. Poly(rA) also competed, although less effectively than the single-stranded DNAs. Double-stranded DNA and the single-stranded DNA oligomer $d(pCpT)_5$ showed no observable competition. Thus the relative affinity of ICP8 for the nucleic acids used in this study is as follows: single-stranded HSV-1 DNA $\simeq$ fd DNA $>$ poly(rA) $\gg$ double-stranded HSV-1 DNA $\simeq$ $d(pCpT)_5$.

Electron microscopy of negatively stained DNA protein complexes. ICP8–single-stranded DNA protein complexes were prepared by mixing ICP8 and φX174 viral DNA in protein/DNA weight ratios of 5:1, 10:1, and 15:1. The complexes were fixed, mounted, and stained as described above. Visualization in the electron microscope indicated no apparent morphological differences in the complexes prepared at the various ratios. Uncomplexed DNA at the

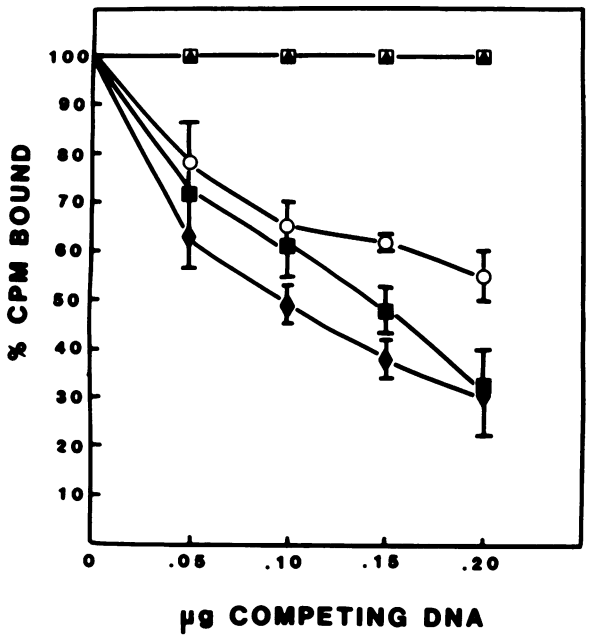

FIG. 4. Results of filter-binding assays carried out in the presence of increasing amounts of unlabeled competing nucleic acids. Points represent the average of duplicate assays in the presence of: ◆, single-stranded HSV-1 DNA; □, double-stranded HSV-1 DNA; ■, fd DNA; ○, poly(rA); ▲, $d(pCpT)_5$.

subsaturating ratio was observed as collapsed, negatively stained tangles under the aqueous conditions used.

A considerable amount of fine structure was revealed by the negative stain (Fig. 5). The average width of the complexes was 9.3 ± 0.8 nm (30 measurements). Portions of the complexes had the appearance of a "beaded necklace" with protein "beads" arranged in a regular tandem array along their length. In contrast, measurements of T4 gene 32 protein-fd complexes prepared and visualized in exactly the same way indicated a width of 5.8 ± 0.6 nm (30 measurements). This value is in good agreement with the previously published value of ~6 nm (2). No clear fine structure was seen along the length of the T4 gene 32 complexes presumably due to the relatively small size (~35,000 daltons) of this DNA-binding protein.

The average width of the individual beads along the axis of the ICP8-fd complexes was 5.3 ± 0.8 nm (25 measurements) and the center-to-center interval was ~9 nm. Determination of the number of these structures along segments of the DNA where they were clearly discernible and extrapolation to the number on a full-length ϕX174 DNA-ICP8 complex results in an estimate of 120 to 160 beads per DNA molecule. The number of ICP8 molecules expected per ϕX174 DNA molecule (5,386 nucleotides; 25) based on the previously established ratio of 40 nucleotides/protein is ~136; hence the observed structures may represent ICP8 monomers.

Stimulation of HSV-1 DNA polymerase in the presence of ICP8. Many of the properties of ICP8 described in this and previous reports (10, 17, 22) are similar to those described for procaryotic single-stranded DNA-binding proteins (11), particularly the T4 gene 32 protein and the *Escherichia coli* single-strand DNA-binding protein (SSB). A feature common to these proteins is their ability to stimulate their homologous DNA polymerases (26). This possibility seemed likely in the case of ICP8 since recent studies by Littler et al. (13) have shown that ICP8 is closely associated with the HSV-1 DNA polymerase and its presence stabilizes the activity of the polymerase. To test whether purified ICP8 was capable of stimulating HSV-1 DNA polymerase activity, polymerase assays were carried out in the presence and absence of ICP8. The reaction mixes contained 50 μg of heat-denatured salmon sperm DNA, 3.5 U of HSV-1 DNA polymerase, the standard deoxynucleotide triphosphate concentrations, and, where appropriate, 0.6 μg of ICP8. The ICP8 used was isolated from the same batch of infected cells

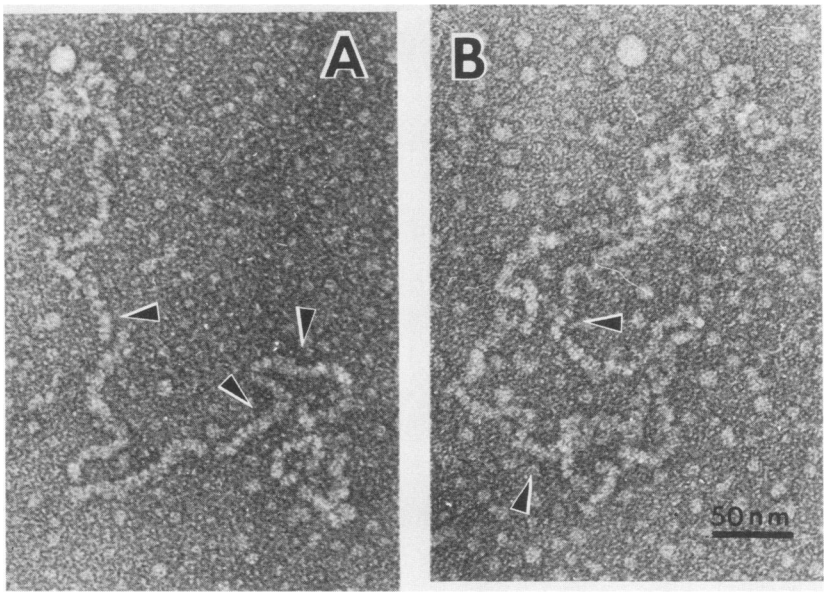

FIG. 5. Electron micrographs of negatively stained ICP8-ϕX174 DNA complexes. (A) Full-length complex linearized by a single break in the DNA; (B) intact circular complex. Arrows indicate regions of the complexes where it is possible to discern individual protein beads. Both complexes were photographed at a magnification of ×80,000.

TABLE 1. Stimulation of HSV-1 DNA polymerase by ICP8

Reaction mix	Polymerase	ICP8	pmol of dTMP incorporated per 30 min
+	+	−	194.6
+	+	+	337.2
+	−	+	<0.1
+	−	−	<0.1

as the polymerase. Typical results are shown in Table 1. The presence of ICP8 resulted in an ~60% increase in incorporation of dTMP under the reaction conditions described. Control reactions done in the absence of the DNA polymerase showed no measurable incorporation. Similar results were obtained with exonuclease III-activated calf thymus DNA (data not shown).

The specificity of the observed stimulation was tested with a heterologous single-strand DNA-binding protein (T4 gene 32 protein) and a heterologous DNA polymerase (calf thymus α). HSV-1 polymerase assays carried out in the presence of 0.6 μg of T4 gene 32 protein resulted in incorporation of [^{3}H]dTMP at 99 ± 3% of control levels. Calf thymus DNA polymerase α reactions in the presence and absence of ICP8 were carried out in parallel with equivalent HSV-1 DNA polymerase control reactions at 50 mM KCl. The presence of 0.6 μg of ICP8 resulted in a 35 to 40% increase in incorporation of dTMP in the polymerase α reactions as compared to a 70% increase in the HSV-1 polymerase reactions.

To determine whether the observed stimulation of the HSV-1 DNA polymerase was due to an increase in the rate of polymerization, time course experiments were carried out in the presence and absence of ICP8. The reaction mixtures contained 2 U of DNA polymerase, 12.5 μg of heat-denatured salmon sperm DNA, and, where appropriate, 0.6 μg of ICP8. Reactions were done in duplicate and stopped at 0, 10, 20, and 30 min. The results are shown in Fig. 6 and indicate that the presence of ICP8 stimulated the rate of polymerization by a factor of ~1.8 under the reaction conditions used.

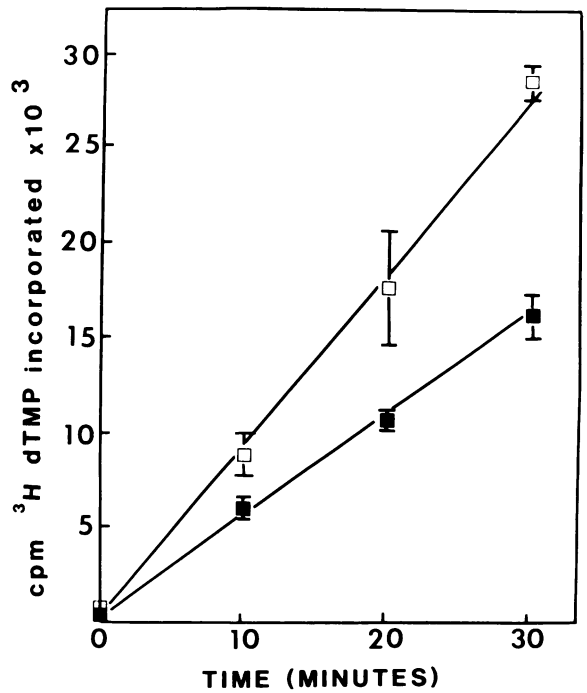

FIG. 6. Time course of HSV-1 DNA polymerase stimulation by ICP8. Symbols: ■, average of duplicate DNA polymerase assays incubated at 37°C for the times indicated; □, averages of duplicate assays carried out in the presence of 0.6 μg of ICP8.

DISCUSSION

The results presented above extend our knowledge of ICP8 at the molecular level and indicate that this protein exhibits a number of properties analogous to those seen with procaryotic DNA-binding proteins. The pH and heat inactivation studies show that ICP8 DNA-binding activity is stable under conditions in which the HSV-1 DNA polymerase also displays maximal activity (moderately high ionic strength, near neutral pH, 37°C). The heat inactivation results also indicate that studies of ICP8 purified from cells infected with temperature-sensitive mutants are feasible at both permissive and nonpermissive temperatures. The pH range of DNA binding is similar to, although more narrow than, that seen with the SSB (24). The fragility of ICP8 to both acidic and basic pH is not unreasonable due to the large size of the polypeptide and the fact that preliminary amino acid analysis of ICP8 indicates the presence of substantial numbers of both acidic and basic residues (W. T. Ruyechan and D. Corcoran, unpublished data).

The competition studies reveal a number of important aspects concerning the interaction of ICP8 with nucleic acids. First, no difference, within the limits of the assay, was observed between the ability of homologous single-stranded HSV-1 DNA (67% guanine plus cytosine) and nonhomologous fd DNA (40% guanine plus cytosine) to compete for ICP8. Thus the single-stranded DNA-binding activity of purified ICP8 does not appear to be sequence or guanine-plus-cytosine content specific. These results do not, however, rule out the possibility of sequence-specific binding of ICP8 mediated by other proteins in the infected cell. Second, purified ICP8 shows a marked preference for single-stranded over double-stranded DNA. These results are in keeping with previous studies involving DNA cellulose chromatography of infected-cell extracts (1, 18, 20). This preference, therefore, appears to be an intrinsic property of the protein. It should be noted that experiments by Powell et al. (17) and work in our own laboratory show that purified ICP8 is capable of binding double-stranded DNA based on filter-binding assays. However, when the binding of labeled, double-stranded HSV-1 DNA is competed by single- and double-stranded HSV-1 DNA and double-stranded λ DNA, single-stranded HSV-1 DNA appears to be approximately fivefold more efficient than either the homologous or the heterologous double-stranded DNAs (W. T. Ruyechan and A. C. Weir, unpublished data). Hence the competition results imply a substantially lower affinity of ICP8 for double-stranded DNA. Third, the inability of d(pCpT)$_5$ to compete for ICP8 in the presence of single-stranded DNA is analogous to results obtained with T4 gene 32 protein and SSB (11, 24). The affinity constants determined for the interaction of these procaryotic proteins with short, single-stranded DNA oligomers were three to five orders of magnitude less than the constants for interaction with long single-stranded DNAs. These large differences are due to the fact that the oligomers were too short to allow cooperative protein-protein interactions to occur. A similar explanation probably applies here since ICP8 has been shown to bind cooperatively to single-stranded DNA (22). Finally, the observation that poly(rA) competes for ICP8 lends support to a model recent-

ly proposed by Godowski and Knipe (5) regarding the regulatory role of ICP8 on HSV-1 gene expression. One aspect of this model involves the binding of ICP8 to mRNA transcripts. The results presented above indicate that ICP8 could certainly interact with a poly(A) tails of such transcripts. The observed competition appears to be specific for single-stranded poly(rA) since filter-binding experiments using heparin as the competing polymer show no effect on the ICP8–single-stranded HSV-1 DNA interaction (Ruyechan and Weir, unpublished data). An ability to bind to its own mRNA transcript is associated with the autoregulation of the T4 gene 32 protein (6, 21).

That no morphological differences were seen between protein-DNA complexes prepared at subsaturating, saturating, and supersaturating concentrations of ICP8 is in agreement with the cooperative nature of ICP8 binding and indicates that the major protein-protein interaction occurs between polypeptides bound adjacently along the DNA strand. The ~9-nm width of the complexes falls between values determined for the widths of T4 gene 32 protein (6 nm)– and SSB (12 nm)–single-stranded DNA complexes (2, 4). If the beads observed in the electron microscope are in fact representations of protein monomers, the indication would be that ICP8 is a relatively asymmetric molecule. Both the T4 gene 32 protein and SSB are asymmetric, with the former protein having an axial ratio of 4:1 (11, 28).

The stimulation of the viral DNA polymerase by ICP8 bears out studies with temperature-sensitive mutants concerning the involvement of ICP8 in the DNA replication process (3, 12, 13, 29). The experiments with T4 gene 32 protein and DNA polymerase α from calf thymus indicate that this stimulation shows at least a limited specificity. No stimulation of the viral polymerase activity was observed in the presence of the T4 gene 32 protein at concentrations which resulted in an ~60% increase in the presence of ICP8. ICP8 did stimulate the eucaryotic polymerase, although the observed stimulation was approximately one-half of the level seen with HSV-1 DNA polymerase. Stimulation of heterologous DNA polymerases by single-strand DNA-binding proteins is not without precedent since, as reviewed by Kowalczykowski et al. (11), the *E. coli* SSB stimulates the bacteriophage T7 DNA polymerase as well as *E. coli* DNA polymerases II and III.

The increased rate of incorporation of nucleotides seen in the presence of ICP8 may indicate that ICP8 holds single-stranded DNA in a configuration which increases the efficiency of the viral DNA polymerase. The observed stimulation also provides a functional assay for purified ICP8. Studies are currently under way to optimize the observed stimulation, using defined single-stranded DNA templates, and to use the ICP8-viral DNA polymerase system as a basic assay for the identification of other proteins involved in the replication of HSV-1 DNA.

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