The UL8 Subunit of the Herpes Simplex Virus Type-1 DNA Helicase-Primase Optimizes Utilization of DNA Templates Covered by the Homologous Single-strand DNA-binding Protein ICP8

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The herpes simplex virus type-1 DNA helicase-primase is a heterotrimer encoded by the UL5, UL8, and UL52 genes. The core enzyme, specified by the UL5 and UL52 genes, retains DNA helicase, DNA-dependent nucleoside triphosphatase, and primase activities. The UL8 subunit has previously been implicated in increasing primer stability and in stimulating primer synthesis by the core enzyme. To further characterize the function of the UL8 subunit, we have examined its effect on the activities of the UL5/52 core enzyme using DNA templates covered by the herpes simplex virus type-1 single-strand DNA-binding protein ICP8. We found that while ICP8 stimulated the DNA helicase activity of the UL5/52 proteins up to 3-fold, maximum stimulation by ICP8 required the presence of UL8 protein. Moreover, UL8 protein was required to reverse the inhibitory effect of ICP8 on the DNA-dependent ATPase and primase activities of the UL5/52 proteins. These observations were specific for ICP8 since the heterologous Escherichia coli single-strand DNA-binding protein could not substitute for ICP8. These data suggest that UL8 protein mediates an interaction between the UL5/52 core enzyme and ICP8 that optimizes the utilization of ICP8-covered DNA templates during DNA replication.

Herpes simplex virus type-1 (HSV-1) is a double-strand DNA virus with a genome of ~152 kilobase pairs and three origins of DNA replication (Challberg, 1991). Replication of origin-containing plasmids requires the action of seven viral gene products (Stow, 1992; Wu et al., 1988). These seven gene products comprise a highly processive heterodimeric DNA polymerase (UL30/UL42 genes), a heterotrimeric DNA helicase-primase (UL5/UL8/UL52 genes), a single-strand DNA-binding protein (SSB) (UL29 gene), and an origin-binding protein (UL9 gene) (reviewed by Challberg (1991)).

HSV-1 SSB, henceforth referred to as ICP8 (infected cell polypeptide 8), is a 128-kDa zinc metalloprotein (Gupte et al., 1991). It is a functional homologue of Escherichia coli SSB (E-SSB) (Lohman and Ferrari, 1994), capable of binding to single-strand DNA cooperatively and with high affinity (Lee and Kisse, 1985; Ruyechan, 1983). The DNA-binding site size for ICP8 is based on indirect measurements. Estimates based on the ratios of ICP8 required for stimulation of the HSV-1 DNA polymerase, strand displacement activity, and nuclease protection fall in the range of one ICP8 to 12–22 nucleotides (Boehmer and Lehman, 1993b; Hernandez and Lehman, 1990; O’Donnell et al., 1987). They are consistent with a site size of 15–18 nucleotides estimated by electron microscopy (Makhov et al., 1996). In addition to its role as an SSB, ICP8 participates in multiple protein-protein interactions that are probably significant during viral DNA replication (Boehmer and Lehman, 1993a; Boehmer et al., 1994; Chiou et al., 1985; Hernandez and Lehman, 1990; Liptak et al., 1996; Lukonis and Weller, 1996; Thomas et al., 1992; Vaughan et al., 1985).

The HSV-1 DNA helicase-primase is thought to be responsible for DNA unwinding and priming of Okazaki fragments at the DNA replication fork (Crute et al., 1989). The core enzyme consists of the 99-kDa UL5 and 114-kDa UL52 gene products and possesses 5’ → 3’ DNA helicase, DNA-dependent nucleoside triphosphatase, and primase activities (Calder and Stow, 1990; Dodson and Lehman, 1991). The primase activity exhibits sequence preference, with the predominant template site in ωX174 DNA being 3’-AGCCCTCCCA, with primer synthesis initiating at the underlined C residue (Tenney et al., 1995). The 80-kDa UL8 gene product lacks detectable enzymatic and DNA binding activities (Dodson and Lehman, 1991; Parry et al., 1993), but has been reported to stimulate primer synthesis by the UL5/52 core enzyme (Tenney et al., 1994). In addition, it has been proposed that UL8 protein increases utilization of primers synthesized by the UL5/52 proteins (Sherman et al., 1992).

This work is a further investigation into the role of the UL8 subunit. We have examined the effects of UL8 protein on the activities of the UL5/52 proteins in the presence of ICP8. Our results show that UL8 protein is required for optimal DNA helicase, DNA-dependent ATPase, and primase activities in the presence of ICP8. Consequently, we propose that UL8 protein mediates an interaction between ICP8 and the UL5/52 proteins, increasing the utilization of ICP8-covered DNA templates.

EXPERIMENTAL PROCEDURES

Materials—Phosphoenolpyruvate (potassium salt) and NADH were purchased from Sigma. [γ-32P]ATP (300 Ci/mmol) and [α-32P]ATP (300 Ci/mmol) were purchased from DuPont NEN and Amersham Corp., respectively. Deoxyribonucleotides and ribonucleotides (ultra-pure) with the exception of ATP were from Pharmacia Biotech Inc. ATP (lithium salt), leupeptin, pepstatin, and phenylmethanesulfonyl fluo-
Function of the HSV-1 DNA Helicase-Primase UL8 Subunit

Proteins—T4 polynucleotide kinase was purchased from New England Biolabs Inc. E. coli SSB was obtained from both Pharmacia Biotech Inc. and Bio-Rad Laboratories Inc. Random hexamer oligodeoxyribonucleotides were purchased from U. S. Biochemical Corp. and Pharmacia Biotech Inc., respectively. Rabbit muscle lactate dehydrogenase and pyruvate kinase, as solutions in 50% glycerol, were obtained from Sigma. Standards for SDS-polyacrylamide gel electrophoresis were obtained from Bio-Rad.

ICP8, UL8 protein, and the UL5/52 core enzyme were purified from total cell extracts of Sf21 cells as described (Skaliter and Lehman, 1989; Stow, 1992).

Stocks of recombinant A. californica nuclear polyhedrosis virus were prepared in S. frugiperda Sf9 cells as described (Skaliter and Lehman, 1989; 5. Sf21 cells were propagated and infected as described (Skaliter and Lehman, 1994). 48 h (UL8 protein and UL5/52 proteins) or 60 h (ICP8) post-infection, cells were harvested, washed in phosphate-buffered saline, and frozen at −80°C. All subsequent manipulations were performed on ice or at 4°C in buffers containing 0.5 μM leupeptin, 0.7 μg/ml pepstatin, and 0.1 mM phenylmethylsulfonyl fluoride. The cells were disrupted by homogenization in buffer A (10 mM Tris-HCl, pH 8.0, 2 mM EDTA, 0.15 M NaCl) and allowed to swell for 20 min. The cells were lysed with 10 strokes of a tight-fitting pestle (A), and NaCl was added to 1.2 M. After a 30-min extraction, the debris was pelleted by centrifugation at 35,000 rpm for 60 min in a Beckman type 42.1 rotor. Supernatants were dialyzed twice against 2 liters of buffer B (20 mM HEPES/NaOH, pH 7.1, 1 mM dithiothreitol, 10% glycerol, and 0.1 mM EDTA) containing 0.1 M (ICP8), 50 mM (UL8 protein), or 60 mM (UL5/52 proteins) NaCl to give fraction I.

All chromatography steps were performed using a Pharmacia FPLC system at a flow rate of 0.5 ml/min. ICP8 was purified as described previously (Boehmer and Lehman, 1993a). At each stage during the purification of UL8 protein and the UL5/52 core enzyme, chromatography fractions were assayed by SDS-polyacrylamide gel electrophoresis followed by Coomassie Blue staining and by immunoblotting using rabbit sera raised against UL8 protein and the UL5/52 core enzyme (courtesy of I. R. Lehman, Stanford University). Final purity was assessed by densitometric scanning of Coomassie Blue-stained SDS-polyacrylamide gels.

UL8 protein fraction I was loaded onto a 5-ml Econo-Pac HTP cartridge (Bio-Rad) equilibrated with buffer C (50 mM imidazole HCl, pH 7.0, 1 mM dithiothreitol, 10% glycerol, and 50 mM NaCl). The column was washed; proteins were eluted with a 50-ml linear gradient of ammonium sulfate (0-390 mM) in buffer C; and 1-ml fractions were collected. Fractions 7-16, containing the peak of UL8 protein, eluting at ~75% ammonium sulfate, were pooled and dialyzed against 2 liters of buffer D (20 mM Tris-HCl, pH 7.5, 1 mM dithiothreitol, 10% glycerol, and 0.1 mM EDTA) containing 50 mM NaCl to give fraction II. Fraction II was applied to a Mono Q HR 5/5 column (Pharmacia Biotech Inc.) equilibrated with buffer D containing 50 mM NaCl. The column was washed, and proteins were eluted with a 10-ml linear gradient of NaCl (0-0.5 M) at 1 ml/min. Fractions containing homogenous (∼95% pure) UL8 protein, eluting at ~0.3 M NaCl, were divided into aliquots, frozen in liquid nitrogen, and stored at −80°C. The yield of purified UL8 protein was ~3.5 mg from 2.5 g of cell paste.

UL5/52 proteins fraction I was loaded onto a 5-ml Hitrap heparin column (Pharmacia Biotech Inc.) equilibrated with buffer B containing 60 mM NaCl. The column was washed; proteins were eluted with a 50-ml linear gradient of sodium phosphate (0-390 mM) in buffer B; and 1-ml fractions were collected. Fractions 13-28, containing the bulk of UL5/52 proteins, were pooled (fraction II) and applied directly to a 5-ml Econo-Pac HTP cartridge equilibrated with buffer E (10 mM sodium phosphate, pH 7.0, 1 mM dithiothreitol, 10% glycerol, and 0.1 M NaCl). The column was washed; proteins were eluted with a 50-ml linear gradient of sodium phosphate (0.01–0.2 M) in buffer E; and 1-ml fractions were collected. The column flow-through fraction and fractions 1–12, containing the majority of UL5/52 proteins, were pooled and dialyzed against 2 liters of buffer D containing 50 mM NaCl to give fraction III. Fraction III was applied to a Mono Q HR 5/5 column equilibrated with buffer D containing 50 mM NaCl. The column was washed, and proteins were eluted with a 10-ml linear gradient of NaCl (0.05–0.4 M) at a flow rate of 1 ml/min. Fractions containing homogenous (∼95% pure) UL5/52 proteins, eluting in the 1 M NaCl step, were divided into aliquots, frozen in liquid nitrogen, and stored at −80°C. The yield of purified UL5/52 proteins was ~4.5 mg from 7.5 g of cell paste.

Protein concentrations were determined by using extinction coefficients of 82,720, 130,390, and 171,380 M−1 cm−1 at 280 nm for ICP8, UL8 protein, and UL5/52 proteins, respectively. Fig. 1. shows the purified ICP8, UL5/52 proteins, and UL8 protein, respectively. The positions of standards and molecular weight species present in the protein preparations were confirmed as proteolytic degradation products by immunoblot analysis.

DNA Substrates—ωX174 virion DNA was purchased from U. S. Biochemical Corp. Random hexamer oligodeoxyribonucleotide primers were obtained from Life Technologies, Inc. Oligodeoxyribonucleotides were synthesized on an Applied Biosystems DNA synthesizer and purified by electrophoresis through denaturing polyacrylamide gels (Sambrook et al., 1989). The DNA helicase substrate was constructed by annealing 5′-2P-labeled 59-mer oligodeoxyribonucleotide (residues 26–59 are complementary to residues 10–74) as a scaffold. The product was purified by electrophoresis through denaturing polyacrylamide gels (Sambrook et al., 1989). The DNA helicase substrate was constructed by annealing 5′-2P-labeled 59-mer oligodeoxyribonucleotide (residues 26–59 are complementary to residues 1–34 of the 90-mer oligodeoxyribonucleotide) to the 90-mer oligodeoxyribonucleotide. This produced a Y-shaped partial DNA duplex molecule that possesses a 25-nucleotide 5′-2P-tail that serves as a loading site for the 5′→3′ translocation of the UL5/52 DNA helicase.

Enzyme Assays—DNA helicase activity was measured by quantitating the displacement of 5′-2P-labeled 59-mer oligodeoxyribonucleotide using the Y-shaped DNA substrate. Reactions (10 μl) were performed at 37°C in 20 mM HEPS/NaOH, pH 7.5, 1 mM dithiothreitol, 10% glycerol, and 4.5 mM MgCl2 containing 3 μM ATP, 0.1 mg/ml bovine serum albumin, 15 nM (molecules) DNA substrate, a 5–10-fold molar excess of unlabeled 27-mer oligodeoxyribonucleotide to prevent reannealing of the unwound DNA strand, and the indicated concentrations of UL5/52 proteins, UL8 protein, ICP8, and E-SSB. In this and subsequent assays, E-SSB was added to achieve the indicated protein/nucleotide ratios assuming a site size of 35 nucleotides/nteramer of protein (Lehman and Ferrai, 1994). The reactions were terminated by the addition of 3 μl of 100 mM EDTA, pH 8.0, 10% SDS, 40% glycerol, and 0.5% bromphenol blue. The reaction mixtures were resolved by electrophoresis through nondenaturing polyacrylamide gels, and DNA unwinding was measured as described previously (Boehmer et al., 1993).

The hydrolysis of ATP to ADP and P 32 was coupled to the oxidation of NADH to NAD + and was measured by a decrease in the absorbance at 340 nm as a function of time (Boehmer and Emmerson, 1992). Reactions (75 μl) were performed at 37°C in 20 mM HEPS/NaOH, pH 7.6, 1 mM dithiothreitol, 10% glycerol, and 3.5 mM MgCl2 containing 1 μM ATP, 200 μM NADH, 1.5 mM phosphoenolpyruvate, 3 units each of lactate dehydrogenase and pyruvate kinase, and 50 nM oligodeoxyribonucleotide PB-10 as cofactor, 25 nM UL5/52 proteins, and the indicated concentrations of UL8 protein, ICP8, and E-SSB. The data were collected with a Perkin-Elmer Lambda 25 spectrophotometer using PECSS software Version 4.2 to calculate rates of ATP hydrolysis.

Primase activity was coupled to the incorporation of [γ-32P]ATP by Sequence Reaction (20 μl) were performed at 30°C in 20 mM EPPS/NaOH, pH 8.3, 1 mM dithiothreitol, 10% glycerol, and 6.5 mM MgCl2 containing 1 μM each ATP, CTP, GTP, and UTP, 40 μM each dCTP, dGTP, and dTTP, 20 μM [γ-32P]ATP (~3500 Ci/mmol), 0.1 mg/ml bovine serum albumin, 1 unit of Sequenase, 1.43 nM (molecules) ωX174 virion DNA as template, and the indicated concentrations of UL5/52 proteins, and UL8 protein, respectively. The positions of standards and molecular weight species present in the protein preparations were confirmed as proteolytic degradation products by immunoblot analysis.
UL8 protein, ICP8, and E-SSB. After 60 min, the reaction mixtures were spotted onto individual discs of DE81 paper (Whatman), followed by three 10-min washes in 0.5 M sodium phosphate, pH 7.0, and one wash in 95% ethanol. The DE81 paper discs were dried, and their radioactivity was determined by scintillation counting.

**RESULTS**

Effects of ICP8, E-SSB, and UL8 Protein on the DNA Helicase Activity of the UL5/52 Proteins—Fig. 2 shows the ability of the UL5/52 proteins to utilize the Y-shaped partial DNA duplex molecule as a substrate for helicase activity. High concentrations of UL5/52 proteins (250 nM) were required to unwind >50% of the DNA substrate. In contrast to the DNA primase activity (Crute and Lehman, 1991), the DNA helicase activity of the UL5/52 proteins was observed to be most active at pH 7.0–7.5, with virtually undetectable levels of activity at pH 8.0–8.6 (data not shown).

Fig. 3 shows the effect of ICP8 on the helicase activity of the UL5/52 proteins. The data indicate that concentrations of ICP8 in excess of those of the UL5/52 proteins (150 nM) could stimulate the helicase activity of the UL5/52 proteins up to 3-fold (Fig. 3A). There was no significant DNA unwinding activity in the presence of ICP8 alone. Fig. 3B shows the stimulatory effect of ICP8 on the kinetics of DNA unwinding by the UL5/52 proteins.

Fig. 4 shows the effect of UL8 protein on the helicase activity of the UL5/52 proteins in the absence or presence of equimolar (300 nM) or excess (450 nM) ICP8. Where indicated, UL8 protein was present at a 3-fold molar excess over the UL5/52 proteins. This ratio had previously been employed by Tenney et al. (1994) to examine the effect of UL8 protein on the primase activity of the UL5/52 proteins. The addition of this level of UL8 protein had no significant effect on the helicase activity of the UL5/52 proteins (Fig. 4). Further titration of UL8 protein showed no effect on DNA unwinding even when present at a 6-fold molar excess over UL5/52 proteins (data not shown).

The addition of UL8 protein to reactions containing UL5/52 proteins and ICP8 markedly increased the level of stimulation of helicase activity observed with ICP8 alone (Fig. 4). While ICP8 concentrations of 300 and 450 nM increased DNA unwinding 1.6- and 3.6-fold, respectively, the presence of UL8 protein boosted this stimulation to 3.1- and 8.6-fold, respectively. Increasing the ratio of UL8 protein to UL5/52 proteins did not significantly modulate the stimulatory effect observed in the presence of ICP8 (data not shown). Fig. 5 shows the synergistic effect of ICP8 and UL8 protein on the kinetics of DNA unwinding by the UL5/52 proteins.

Fig. 6 shows that heterologous E. coli SSB could not substitute for ICP8 in this synergistic response. The addition of UL8 protein to reactions containing 300 nM ICP8 stimulated DNA unwinding by the UL5/52 proteins from 2.3- to 5.6-fold. In contrast, equimolar concentrations of E-SSB led to inhibition of DNA unwinding by the UL5/52 proteins, both in the absence and presence of UL8 protein. The additive effect of ICP8 and UL8 protein on the helicase activity of the UL5/52 proteins was also observed using a DNA substrate in which a 3′-tailed oligodeoxyribonucleotide was annealed to single-strand M13 DNA (data not shown). The results show representative data that were reproducibly observed in three independent experiments.

### Table I

Oligodeoxyribonucleotides used in this study

| Name | Sequence |
|------|----------|
| PB-10 | 5′-GTACCGGGGATCCTAGTGAGGCTGCATGGTACGCGGCCGTTTTGCGTACCG |
| 59-mer | 5′-GGTGTTGTGGATGTCGCTGCGAGGCTGCATGGTACGTATC |
| 90-mer | 5′-GGTGTGGATGTCGCTGCGAGGCTGCATGGTACGCGGCCGTTTTGCGTACCG |

**Fig. 2.** DNA unwinding of the Y-shaped partial DNA duplex molecule by the UL5/52 proteins. Reactions were performed as described under “Experimental Procedures” for 60 min with the indicated concentrations of UL5/52 proteins. A, autoradiogram of the reaction products. Lane 1, no protein; lanes 2–7, 12.5, 25, 50, 100, 250, and 500 nM UL5/52 proteins, respectively; lane 8, heat-denatured DNA substrate. B, quantitation of the data shown in A.

Effects of ICP8, E-SSB, and UL8 Protein on the DNA-dependent ATPase Activity of the UL5/52 Proteins—The dependence of helicase action on ATP hydrolysis led us to examine the effects of ICP8, E-SSB, and UL8 protein on the DNA-dependent ATPase activity of the UL5/52 proteins. Under the conditions used in this study, DNA-dependent ATPase hydrolysis by the UL5/52 proteins proceeded with linear kinetics (Fig. 7). When included, UL8 protein was present at a 3-fold molar excess over the UL5/52 proteins.

Fig. 8 shows the effects of ICP8, E-SSB, and UL8 protein on the rate of ATP hydrolysis by the UL5/52 proteins. There was no significant effect on the rate of ATP hydrolysis in the presence of UL8 protein. In contrast, ICP8 or E-SSB caused significant inhibition, depending on the protein/nucleotide ratio. An ICP8/nucleotide ratio of 1.75, which is in excess of the concentration required to coat the DNA template, reduced the rate of ATP hydrolysis to 36%. At an ICP8/nucleotide ratio of 1:15, the rate of ATP hydrolysis was reduced to 75%. Similarly, a reduction in the rate of ATP hydrolysis was observed at E-SSB concentrations that were in excess or sufficient to coat the DNA template. Thus, E-SSB/nucleotide ratios of 1:7.5 and 1:3 reduced the rate of ATP hydrolysis to 26 and 41%, respectively. The addition of UL8 protein to reactions containing ICP8 resulted in partial or complete restoration of the rate of ATP hydrolysis, depending on the concentration of ICP8. The addi-
tion of UL8 protein at an ICP8/nucleotide ratio of 1:7.5 increased the rate of ATP hydrolysis to 51%. Complete (95%) restoration of the rate of ATP hydrolysis was observed in the presence of UL8 protein at an ICP8/nucleotide ratio of 1:15. In contrast, the addition of UL8 protein to reactions containing E-SSB did not increase the rate of ATP hydrolysis. The ability of UL8 protein to increase the rate of ATP hydrolysis in reactions inhibited by the addition of ICP8, but not in those with E-SSB, was also observed at lower protein/nucleotide ratios (data not shown). These effects were observed in several independent experiments.

Effects of ICP8, E-SSB, and UL8 Protein on the Primase Activity of the UL5/52 Proteins—In this study, the primase activity of the UL5/52 proteins was measured using a coupled assay in which RNA primers were extended by Sequenase DNA polymerase using single-strand φX174 DNA as template. To determine the effects of ICP8, E-SSB, and UL8 protein on primase activity, it was imperative to exclude any secondary effects of these proteins on Sequenase activity. Table II shows that UL8 protein had no significant effect on the DNA polymerase activity of Sequenase using single-strand φX174 DNA primed with random hexamer oligodeoxyribonucleotides. In contrast, the addition of ICP8 or E-SSB reduced DNA synthesis to 53 and 75% of the normal level, respectively. Similar results were obtained when using singly primed M13 DNA as template instead of random-primed φX174 DNA (data not shown). The concentrations of ICP8 and E-SSB used were in excess of those required to coat the DNA template, representing protein/nucleotide ratios of 1:15 and 1:17.5, respectively, and were the highest concentrations used in the primase assays. The magnitudes of the effects of ICP8 and E-SSB on Sequenase activity cannot account for their strong inhibition of UL5/52 primase-dependent DNA synthesis (see Figs. 9 and 10). Consequently, it is reasonable to assume that the observed effects of ICP8, E-SSB, and UL8 protein are due to modulation of primase activity and not of Sequenase activity.

Figs. 9 and 10 show the effects of ICP8 and E-SSB on the...
Primase activity of the UL5/52 proteins in the absence and presence of UL8 protein. Where indicated, UL8 protein was present at a 3-fold molar excess over the UL5/52 proteins. Consistent with the observations of Tenney et al. (1994), a 3-fold molar excess of UL8 protein could stimulate the primase activity of the core enzyme up to 3-fold (see Fig. 10).

Fig. 9 shows that while UL8 protein alone could stimulate primase activity by a factor of 1.3, ICP8 or E-SSB caused significant inhibition, depending on the protein/nucleotide ratio. An ICP8/nucleotide ratio of 1:15, which is sufficient to coat the DNA template, resulted in almost complete inhibition, with 7% residual activity. In contrast, ICP8/nucleotide ratios of 1:30 and 1:60 resulted in much lower levels of inhibition, with 56 and 78% residual activity, respectively. Virtually complete inhibition of primase activity was observed at E-SSB concentrations that were in excess or sufficient to coat the DNA template.

Thus, E-SSB/nucleotide ratios of 1:17.5 and 1:35 resulted in 4 and 7% residual activity, respectively. The inhibitory effect of E-SSB was less severe at a ratio of 1:70, with 27% residual activity. The addition of UL8 protein to reactions containing ICP8 resulted in a partial or complete reversal of the inhibition, depending on the concentration of ICP8. At ICP8/nucleotide ratios of 1:15 and 1:30, primase activity was increased to 24 and 74%, respectively. The inhibitory effect of E-SSB was less severe at a ratio of 1:70, with 27% residual activity. The addition of UL8 protein to reactions containing E-SSB was less severe at a ratio of 1:70, with 27% residual activity. The partial UL8 protein-dependent rescue from inhibition, observed at all concentrations of UL5/52 proteins examined (Fig. 10), Titration of the molar ratio of UL8 protein to UL5/52 proteins from 0.5 to 15:1 did not enhance the rescue from inhibition by ICP8 (data not shown).
helicase activity of the UL5/52 proteins. These observations are consistent with data from other laboratories (Calder and Stow, 1990; Dodson and Lehman, 1994). UL8 protein could stimulate reactions containing E-SSB. The results reflect the average of two separate experiments.

**DISCUSSION**

We have examined the effect of UL8 protein on the utilization of SSB-covered DNA templates by the UL5/52 DNA helicase-primase core enzyme. In the absence of HSV-1 SSB, ICP8, or E-SSB, UL8 protein had no significant effects on the DNA helicase or DNA-dependent ATPase activities of the UL5/52 proteins. These observations are consistent with data from other laboratories (Calder and Stow, 1990; Dodson and Lehman, 1991). In addition, as previously reported by Tenney et al. (1994), UL8 protein could stimulate the primase activity of the UL5/52 proteins 3-fold.

In the absence of UL8 protein, ICP8 stimulated the DNA helicase activity of the UL5/52 proteins 3-fold. This observation is not surprising since SSBs frequently stimulate DNA unwinding (Kornberg and Baker, 1992; Lehman and Bjornson, 1996). The stimulatory effect of ICP8 was specific since heterologous E-SSB could not substitute for ICP8, suggesting that ICP8 is not merely stabilizing unwound regions of DNA. Consequently, the ability of ICP8 to stimulate the DNA helicase activity of the UL5/52 proteins is indicative of a specific physical interaction between these proteins. Consistent with our finding, Crute and Lehman (1991) had previously reported the specific requirement for ICP8 in the unwinding of nicked plasmids by the HSV-1 DNA helicase-primase holoenzyme.

The primase and DNA-dependent ATPase activities of the UL5/52 core enzyme were strongly inhibited by ICP8 or E-SSB in a manner that varied with the SSB/nucleotide ratio. This observation may be rationalized by assuming that SSBs prevent access of the UL5/52 proteins to the DNA template.

The addition of UL8 protein to reactions containing ICP8 and the UL5/52 core enzyme led to further stimulation of DNA unwinding and to reversal of inhibition of the primase and DNA-dependent ATPase activities. These effects were specific for ICP8-covered DNA templates since UL8 protein failed to stimulate reactions containing E-SSB.

The DNA helicase-primase holoenzyme purified from HSV-1-infected cells consists of a 1:1:1 complex of the UL5, UL8, and UL52 subunits (Crute and Lehman, 1991). However, it is unclear what the relative ratios of these proteins are in vivo. The only data regarding this issue are that the UL8 transcript (Baradaran et al., 1994) and protein (Olivo et al., 1989), like those for the UL5 and UL52 genes, are of relatively low abundance. In our experiments, UL8 protein was added to the core enzyme to reconstitute the heterotrimeric holoenzyme. Tenney et al. (1994) reported that optimal in vitro reconstitution of the holoenzyme was achieved at a molar ratio of UL8 protein to UL5/52 proteins of 3:1. Accordingly, in our experiments, UL8 protein was present at a 3-fold molar excess over the UL5/52 proteins. In vivo, we envisage that the UL8/52 heterotrimer interaction with ICP8-covered DNA templates would not require an excess of UL8 protein.

We propose that UL8 protein functions in a manner analogous to the bacteriophage T4 gene 59 protein and the E. coli DnaC protein. The T4 gene 59 protein has been reported to interact with T4 SSB (gene 32 protein)-coated single-strand DNA, and the DNA helicase component (gene 41 protein) of the T4 DNA helicase-primase (gene 41/61 proteins) (Barry and Alberts, 1994a, 1994b; Morrical et al., 1994; Yonesaki, 1994). Consequently, its proposed role is to facilitate loading of the T4 DNA helicase-primase onto DNA templates coated with gene 32 protein to initiate lagging-strand DNA synthesis. The situation during E. coli replication is more complex (reviewed by Kornberg and Baker (1992)). In this system, the DnA protein loads the DnaB helicase, and subsequently the DnaG primase, onto E-SSB-coated DNA templates, either in reactions that require DnaA protein and a DnaA protein recognition site or in combination with the primosome proteins PriA, PriB, and PriC and a primosome assembly site.

Based on the ability of UL8 protein to mediate a functional interaction between ICP8 and the UL5/52 core enzyme, we hypothesize that there may be a direct interaction between...
these proteins. In the physiological state, during DNA replication, this interaction would optimize the utilization of ICP8-covered DNA templates. Our assumption of the existence of protein-protein interactions between the subunits of the HSV-1 DNA helicase-primase and ICP8 is supported by the finding that anti-ICP8 rabbit serum can immunoprecipitate a protein complex that contains the HSV-1 DNA helicase-primase holoenzyme, the heterodimeric DNA polymerase, and ICP8 (Skaliter and Lehman, 1994).

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The UL8 Subunit of the Herpes Simplex Virus Type-1 DNA Helicase-Primase Optimizes Utilization of DNA Templates Covered by the Homologous Single-strand DNA-binding Protein ICP8

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