Journal of General Virology (1997), **78**, 857–865. Printed in Great Britain

A functional interaction of ICP8, the herpes simplex virus single-stranded DNA-binding protein, and the helicase–primase complex that is dependent on the presence of the UL8 subunit

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The herpes simplex virus type 1 (HSV) single-stranded DNA-binding protein (SSB, ICP8) stimulates the viral DNA polymerase (Pol) on an oligonucleotide-primed single-stranded DNA template. This stimulation is non-specific since other SSBs also increase Pol activity. However, only ICP8 was stimulatory when Pol activity was dependent upon priming by the viral helicase–primase complex. ICP8 also specifically stimulated the primer synthesis and ATPase activities of the helicase–primase. The mechanism of stimulation was different from that of Pol; helicase–primase stimulation required much lower amounts of ICP8 than the amount that saturates the DNA and optimally stimulates Pol. Furthermore, ICP8 did not act by removing secondary structure as stimulation also occurred on homopolymer templates. While the UL8 component of the helicase–primase is not required for enzymatic activities by a subassembly of the UL5 and UL52 proteins, only the holoenzyme (UL5/8/52) was stimulated by ICP8. These results identify a unique, functional interaction between the ICP8 SSB and the helicase–primase complex, mediated by the UL8 subunit.

Introduction

Herpes simplex virus type 1 (HSV) encodes seven genes required for viral DNA synthesis and for replication of origin-containing plasmid DNA (reviewed in Challberg, 1991; Weller, 1991). Biochemical analysis of the purified gene products has allowed their enzymatic functions to be delineated. The UL9 gene encodes a protein that binds specifically to the origins of HSV DNA replication (Elias *et al.*, 1986; Olivo *et al.*, 1988; Weir *et al.*, 1989), facilitating its unwinding (Makhov *et al.*, 1996). The UL30 gene product is the DNA polymerase catalytic subunit (Pol; Purifoy *et al.*, 1977) and the product of the UL42 gene forms a complex with Pol and increases its processivity (Gallo *et al.*, 1989; Gottlieb *et al.*, 1990; Hernandez & Lehman, 1990; Hamatake *et al.*, 1993). The products of the UL5, UL8 and UL52 genes form a heterotrimeric complex which has both helicase and primase activities (Crute *et al.*, 1988, 1989; Crute & Lehman, 1991). Helicase and primase activity is also observed with a subassembly composed of the UL5 and UL52 proteins (Calder & Stow, 1990; Dodson & Lehman, 1991; Tenney *et al.*, 1994). The sequence of the UL5 protein (McGeoch *et al.*, 1988) has revealed that it belongs to a superfamily of known and putative helicases (Gorbalenya *et al.*, 1989) and the primase active centre of the complex has been localized within the UL52 subunit (Klinedinst & Challberg, 1994; Dracheva *et al.*, 1994). Recent work has shown that mutation of the conserved helicase domains of UL5 disrupts helicase activity while preserving primase activity (K. L. Graves-Woodward & S. K. Weller, unpublished observations). While UL8 alone does not bind to nucleic acid (Parry *et al.*, 1993), it has been proposed to function in transport of the UL5/52 subassembly to the nucleus (Calder *et al.*, 1992). Additionally, UL8 forms a complex with the UL9 protein (McLean *et al.*, 1994). These functions do not directly implicate UL8 in helicase or primase enzymatic activity. However, the activity of the UL5/52 subassembly is stimulated by UL8

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(Sherman *et al.*, 1992) at the level of primer synthesis (Tenney *et al.*, 1994, 1995) so that its involvement in catalysis may be more dynamic than previously believed. The final gene required for viral DNA replication is the UL29 gene, which encodes ICP8, a single-stranded DNA-binding protein (SSB) (Bayliss *et al.*, 1975; Powell *et al.*, 1981). The ICP8 SSB has been shown to stimulate Pol activity (Ruyechan & Weir, 1984; Hernandez & Lehman, 1990). Stimulation is thought to occur through elimination of secondary structure in the template. This function is nonspecific in that heterologous *E. coli* SSB has also been shown to stimulate Pol (O'Donnell *et al.*, 1987). In contrast, ICP8, and not other SSBs, specifically stimulates the helicase activity of the UL9 protein (Fierer & Challberg, 1992; Boehmer *et al.*, 1993). The UL9/ICP8 interaction is further characterized by a tight binding between the two proteins (Boehmer & Lehman, 1993; Boehmer *et al.*, 1994).

We have used a coupled primase–polymerase DNA synthesis system similar to that described by Sherman *et al.* (1992) composed of unprimed ϕX174 DNA, Pol/UL42, UL5/52, UL8 and ICP8. Pol activity in this system is dependent upon oligoribonucleotide primer synthesis by UL5/8/52 and is stimulated by ICP8. Although heterologous *E. coli* SSB and T4 gene 32 SSB stimulated Pol activity on an oligonucleotide-primed template, they did not substitute for ICP8 in the coupled primase–polymerase DNA synthesis system. The amount of ICP8 required for stimulation in the coupled primase–polymerase system was markedly lower than the amount required for stimulation of Pol, suggesting that ICP8 affected primase rather than Pol in the coupled system. Direct examination of oligoribonucleotides synthesized by UL5/8/52 showed that ICP8 stimulated primer synthesis. ICP8 also stimulated the DNA-dependent nucleoside 5′-triphosphatase (NTPase) activity of the UL5/8/52 complex. The activities of the UL5/52 subassembly, in contrast, were not stimulated by ICP8. These results suggest the existence of a functional interaction between ICP8 and the UL8 subunit.

Methods

■ **Nucleic acids.** Single-stranded ϕX174 and M13 virion DNAs were obtained from New England Biolabs and GIBCO BRL, respectively. Polydeoxycytidine polydeoxyadenosine and polydeoxythymidine were obtained from Pharmacia. Oligonucleotides were obtained from Genosys Biotechnologies.

■ **Expression and purification of recombinant proteins.** HSV DNA replication proteins were expressed by recombinant baculovirus infection of Sf9 insect cells (Invitrogen) as described previously (Tenney *et al.*, 1994). Baculoviruses expressing the HSV UL5, UL8, UL52 (Dodson *et al.*, 1989) and UL30 (Pol; Hernandez & Lehman, 1990) proteins were a generous gift of I. Robert Lehman. The baculoviruses expressing the HSV UL42 (Hamatake *et al.*, 1993) and ICP8 (Tenney *et al.*, 1994) proteins were described previously.

Purification of the recombinant Pol/UL42 complex (Hernandez & Lehman, 1990), and of ICP8, the UL5/52 subassembly and UL8 (Tenney *et al.*, 1994) was described previously. Protein concentrations were determined by absorbance measurements at 280 nm using calculated extinction coefficients (Gill & von Hippel, 1989) based on predicted amino acid sequences. Purified proteins were visualized by denaturing PAGE (Laemmli gels) followed by staining in Coomassie brilliant blue using standard techniques.

■ **Coupled primase–polymerase DNA synthesis system.** Primase-dependent Pol activity was assayed as previously described (Tenney *et al.*, 1994). Reactions contained 50 mM NaCl, 50 mM Tris–HCl (pH 8·0), 1 mM dithiothreitol (DTT), 200 μg/ml acetylated BSA, 10% (w/v) glycerol, 1 mM ATP, 1 mM GTP, 0·1 mM CTP, 0·1 mM UTP, 50 μM dATP, dCTP and dGTP, 5 μM dTTP, 0·22 μCi [^{3}H]dTTP (78 Ci/mmol; New England Nuclear), 4 mM $MgCl_2$ and 50 ng ϕX174 virion DNA (28·6 fmol circular molecules). Pol/UL42, ICP8, UL5/52 and UL8 were added as indicated. Reactions (total volume 50 μl) were incubated at 30 °C for 2 h, and were terminated by trichloroacetic acid precipitation as described (Hamatake *et al.*, 1993), followed by scintillation counting. To visualize products (Figs 1 and 2), reactions were performed with [α-^{32}P]dATP and terminated by the addition of 10 μl gel loading buffer containing 5% SDS. Products were resolved by electrophoresis in native 0·8% agarose, Tris–borate–EDTA (TBE) horizontal slab gels, followed by autoradiography.

In reactions using oligonucleotide-primed ϕX174 DNA as a template, an oligonucleotide corresponding to ϕX174 nucleotide numbers 145–183 (GenEMBL database accession number J02482) was pre-annealed to ϕX174 virion DNA.

■ **Direct oligoribonucleotide primer synthesis assay.** Primer synthesis assays were as described previously (Tenney *et al.*, 1994) in 10 μl reactions with the same reaction components as those for coupled primase–polymerase assays except for the following: UTP was 0·04 μCi/μl [α-^{32}P]UTP (NEN DuPont; 3000 Ci/mmol, 10 μCi/μl), dNTPs were omitted and ϕX174 virion DNA was used at 30 μg/ml nucleotide concentration (17 nM circular molecules) except where noted. Reactions were incubated for 60 min at 30 °C and terminated by adding formamide to 50%. Reaction products were resolved by electrophoresis in denaturing 20% polyacrylamide, 7 M urea, TBE gels and visualized by autoradiography.

■ **DNA-dependent ATPase.** Assays for DNA-dependent ATPase activity were modified from the method of Crute *et al.* (1988) and contained 50 mM Tris–HCl (pH 8), 10% (w/v) glycerol, 2 mM ATP, 4 mM $MgCl_2$, 1 mM DTT and 10–20 μg/ml M13 virion effector DNA (unless indicated) in a 50 μl total volume in 96-well microtitre plates. After incubation for 30 min at 30 °C, the hydrolysis of ATP was determined by adding 20 μl of the reaction mixture to 300 μl of 0·03375% (w/v) malachite green, 0·3% ammonium molybdate. Inorganic phosphate released was determined by absorbance at 620 nm (Lanzetta *et al.*, 1979).

Results

Effect of SSBs on HSV DNA polymerase activity in a coupled primase–polymerase system

A coupled primase–polymerase system was established in order to examine potential protein–protein interactions occurring during lagging strand DNA synthesis. This system is similar to one described previously (Sherman *et al.*, 1992) and utilizes ϕX174 DNA, Pol/UL42, UL5/52, UL8 and ICP8. The proteins used in this system were purified from recombinant-

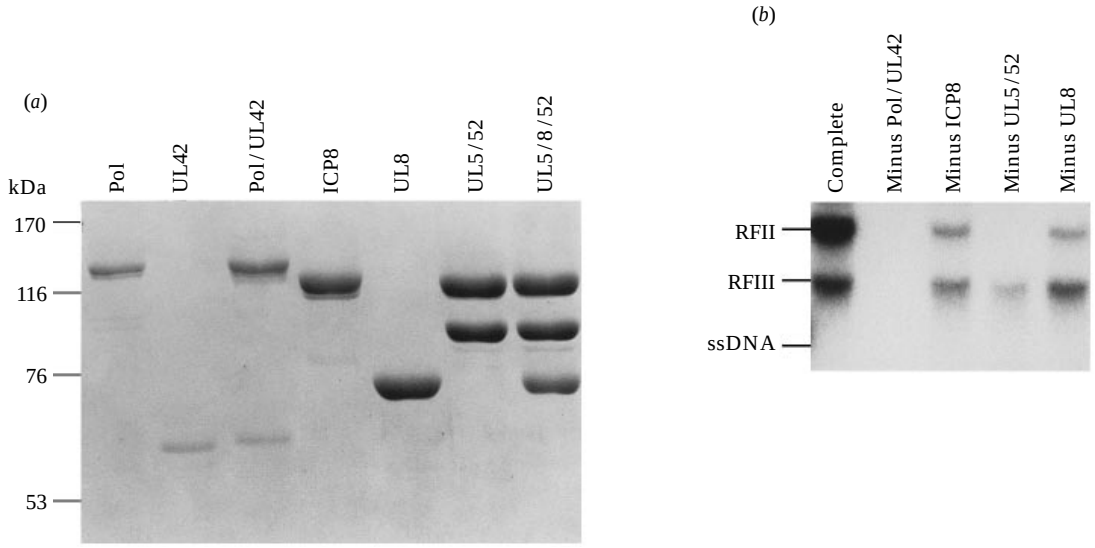

Fig. 1. (*a*) SDS–PAGE of purified HSV-1 replication proteins. Two μg Pol, 1·6 μg UL42, 4 μg Pol/UL42, 2 μg ICP8, 2 μg UL8, 4 μg UL5/52 and 4·5 μg UL5/8/52 were analysed by SDS–PAGE using a 5% stacking gel and 7·5% resolving gel followed by staining with Coomassie Blue. (*b*) Neutral agarose gel analysis of coupled primase–polymerase assay products. Coupled primase–polymerase assays were performed as described in Methods with the indicated omissions. The complete reaction contained 180 fmol Pol/UL42, 880 fmol ICP8, 190 fmol UL5/52 and 300 fmol UL8. The reactions were terminated by the addition of EDTA to 50 mM and aliquots were analysed by electrophoresis in 1% agarose–TBE buffer. The agarose gel was fixed in 10% TCA, dried onto DE81 paper and autoradiographed.

baculovirus-infected cells and are shown in Fig. 1(*a*). Incubation of these proteins with single-stranded circular ϕX174 DNA as described in Methods resulted in oligoribonucleotide synthesis by primase and extension of the primers by Pol/UL42. The DNA reaction products are shown in Fig. 1(*b*). Also shown in Fig. 1(*b*) are products that result from omission of each of the individual protein components. The band migrating at the position of the RFII marker is the product resulting from extension by Pol/UL42 of primers synthesized on single-stranded circular DNA. The band migrating at the position of the RFIII marker is linear double-stranded DNA. This product arises from self-priming of linearized single-stranded DNA. Not surprisingly, incorporation of label into this background RFIII band as well as the RFII band is dependent upon the presence of the Pol/UL42 complex. The RFII product is absolutely dependent upon the presence of the UL5/52 subassembly indicating that it results from primer synthesis. Omission of ICP8 or UL8 resulted in a reduction, but not the complete elimination, of the RFII product. Pol/UL42 and the UL5/52 subassembly are thus capable of carrying out the minimal reactions required for lagging strand DNA synthesis but this process is stimulated by ICP8 and UL8. In previous reports we showed that UL8 stimulates primer synthesis by the UL5/52 subassembly (Tenney *et al.*, 1994, 1995). The mechanism of ICP8 stimulation in the coupled primase–polymerase system could be due to stimulation of primer synthesis or, since ICP8 is known to stimulate Pol/UL42, by affecting Pol/UL42 activity. The effect of ICP8 was investigated further in order to determine its role in the coupled primase–polymerase system.

Shown in Fig. 2 are the products resulting from extension by Pol/UL42 of various primers on the ϕX174 template DNA in the presence of the indicated amounts of ICP8, *E. coli* SSB or T4 gene 32 protein. In Fig. 2(*a*) Pol/UL42 reactions were primed with an oligonucleotide annealed to ϕX174 DNA at a unique site. In Fig. 2(*b*) the reactions were identical to Fig. 2(*a*) except that the template was primed by the UL5/52 subassembly. Similarly, in Fig. 2(*c*) the reactions were primed by UL5/8/52. Fig. 2(*a*) shows that all three SSBs stimulated HSV Pol/UL42 activity on ϕX174 DNA primed at a unique site as seen by the increase in RFII product resulting from full-length extension of primers. The fact that heterologous SSBs stimulate Pol/UL42 activity on oligonucleotide-primed ϕX174 DNA suggests that the mechanism of stimulation is non-specific.

The effect of the SSBs on the UL5/8/52-primed reactions was quite different from the oligo-primed reactions (Fig. 2*c*). At the lower range of ICP8 concentrations, where very little DNA polymerase stimulation was observed in the oligo-primed reactions (Fig. 2*a*, lanes 2–4), there was a marked increase in the amount of RFII product in the UL/5/8/52-primed reactions (Fig. 2*c*) suggesting that the increased incorporation was due primarily to the stimulation of primase activity rather than of polymerase activity. The heterologous SSBs also had a different effect in the UL5/8/52-primed reactions. Whereas in the oligo-primed reactions the heterologous SSBs were stimulatory at the highest amounts tested, these amounts inhibited in the UL5/8/52-primed reactions. At intermediate levels, the heterologous SSBs, unlike the reactions containing ICP8, did not substantially increase the RFII amounts in the

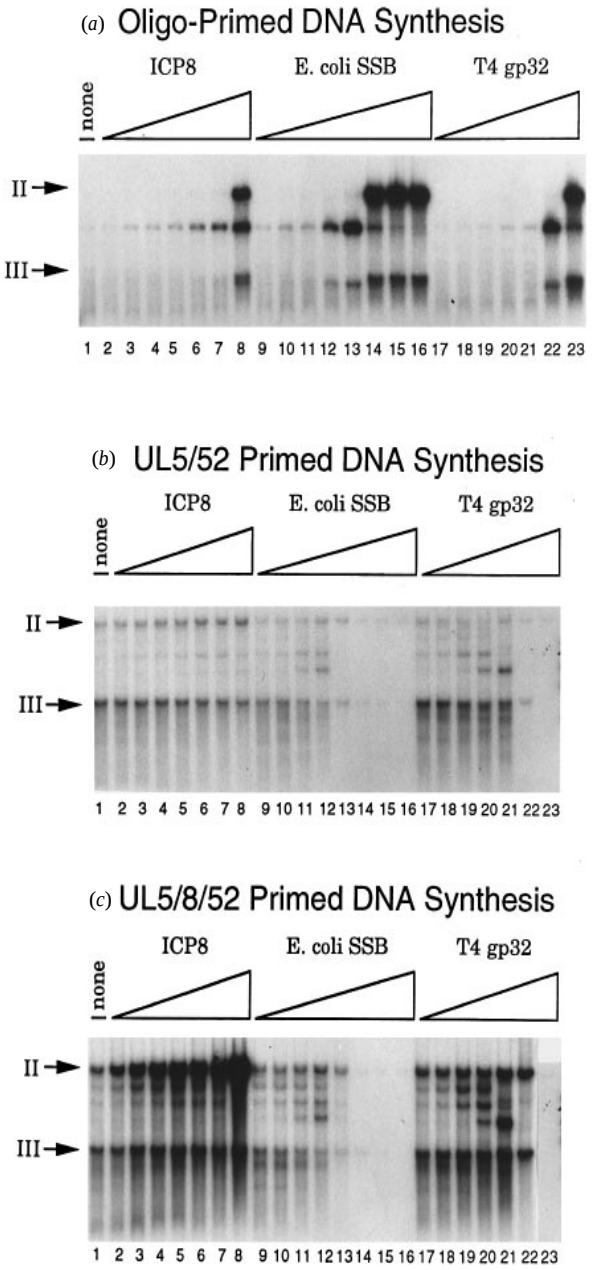

Fig. 2. Effect of SSBs on Pol activity using ϕX174 DNA primed with an oligonucleotide or by HSV-1 primase. (*a*) The oligo-primed DNA synthesis reactions utilized ϕX174 DNA primed with an oligonucleotide complementary to nucleotides 145–183 of ϕX174. The reactions contained 200 fmol Pol/UL42, 150 pmol ϕX174 DNA, the indicated amounts of SSBs, the buffer components described in Methods, and were labelled with [α-^{32}P]dATP. (*b*) UL5/52-primed DNA synthesis reactions utilized unprimed ϕX174 DNA, 190 fmol UL5/52, 200 fmol Pol/UL42, and the same amounts of SSBs used in (*a*). (*c*) UL5/8/52-primed reactions were similar to those in (*b*) except that 490 fmol UL8 was added to UL5/52 to reconstitute UL5/8/52. Lane 1, no SSB; lanes 2–8, 0·11, 0·22, 0·44, 0·88, 1·76, 3·52 and 8·8 pmol ICP8; lanes 9–16, 0·1, 0·2, 0·4, 0·8, 1·6, 3·2, 6·4 and 16 pmol (tetramers) *E. coli* SSB; lanes 17–23, 0·4, 0·8, 1·6, 3·2, 6·4, 12·8 and 31 pmol T4 gene 32 protein.

UL5/8/52-primed reactions. Stimulation in the coupled primase–polymerase system was therefore specific for ICP8. Interestingly, ICP8 did not stimulate RFII production in the UL5/52-primed reactions (Fig. 2*b*) suggesting that the ICP8 stimulation of UL5/8/52 occurred through an interaction with the UL8 subunit.

ICP8 stimulates primer synthesis by UL5/8/52 but not UL5/52

Because of the effect of ICP8 in the coupled primase–polymerase system, we wished to determine whether ICP8 directly affects primer synthesis. Shown in Fig. 3 are ^{32}P-labelled oligoribonucleotides synthesized by UL5/52 or UL5/8/52 in the presence of the indicated amounts of SSBs. The position of the primers synthesized by UL5/52 and UL5/8/52 is indicated by the arrow. We have previously shown that these oligomers are primers based on their ability to be extended by Pol/UL42 (Tenney *et al.*, 1994). As can be seen in Fig. 3, ICP8 stimulated the production of primers by UL5/8/52. On the other hand, ICP8 had no effect on primer synthesis by an equivalent amount of UL5/52. Heterologous SSBs had no effect on either UL5/52 or UL5/8/52. The amounts of the predominant primer synthesized in the presence of UL8 are approximately 3-fold greater than with UL5/52 alone when analysed by quantitative densitometry (data not shown). The addition of ICP8 to UL5/8/52 causes a further 3-fold increase for a total increase of about 9-fold over that of UL5/52 alone. These results suggest that stimulation in the coupled primase–polymerase system originated, at least in part, from stimulation of primer synthesis.

Effect of ICP8 on UL5/8/52 NTPase activity

In addition to primase activity, the UL5/8/52 complex also has helicase and DNA-dependent NTPase activities. We wished to determine if the stimulation of UL5/8/52 by ICP8 was specific for the primase activity or if the interaction affected UL5/8/52 in a more general manner. The effect on ATPase activity was determined since ICP8 could potentially aid in unwinding DNA by binding to the displaced strand and therefore stimulate helicase activity without directly interacting with UL5/8/52. Stimulation of ATP hydrolysis in this case would suggest a direct interaction between ICP8 and UL5/8/52 rather than an indirect effect from the strand displacement reaction. Shown in Fig. 4 are the effects of ICP8, *E. coli* SSB and T4 gene 32 protein on the DNA-dependent ATPase activity of UL5/52 (Fig. 4*a*) and UL5/8/52 (Fig. 4*b*). The data are presented as the percentage activity vs the number of nucleotides per SSB monomer for ICP8 and T4 gene 32 protein or per tetramer for *E. coli* SSB: 100% activity is the amount of ATPase activity on the single-stranded effector DNA in the absence of the SSBs. The DNA-dependent ATPase activities of both the UL5/52 subassembly and the UL5/8/52

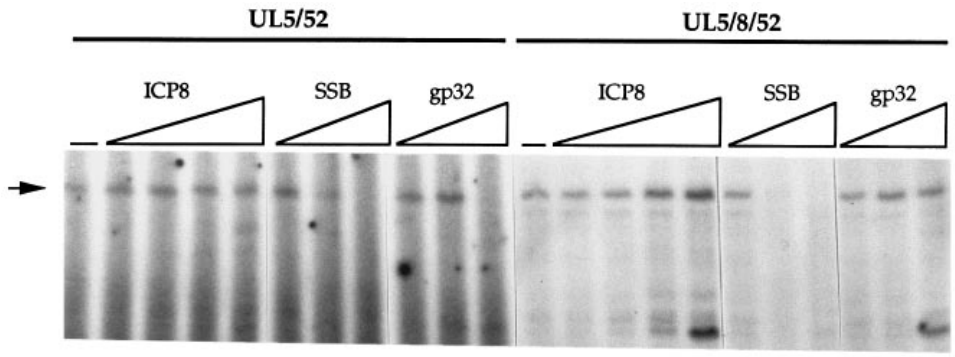

Fig. 3. Effect of SSBs on UL5/52 and UL5/8/52 primer synthesis. Primer synthesis reactions and analyses were as described in Methods. Reactions contained 400 ng ϕX174, 1·88 pmol of the UL5/52 subassembly without (−) or with (+) 5·6 pmol UL8 added. Reactions with ICP8 contained 0, 0·01, 0·1, 1 and 10-fold molar amounts of ICP8 relative to UL5/52. *E. coli* SSB and T4 gp32 contained 0·1, 1 and 10-fold molar amounts relative to UL5/52. The position of the predominant oligoribonucleotide primer is indicated with an arrow. The autoradiographic exposure of the UL5/52 reaction samples is greater than that of the UL5/8/52 samples to enable their visualization.

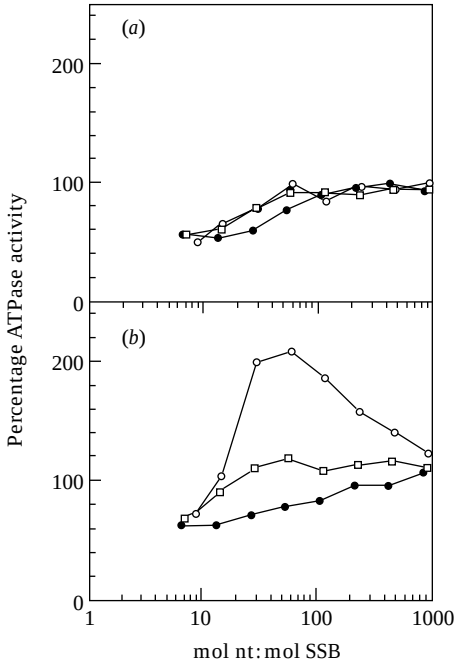

Fig. 4

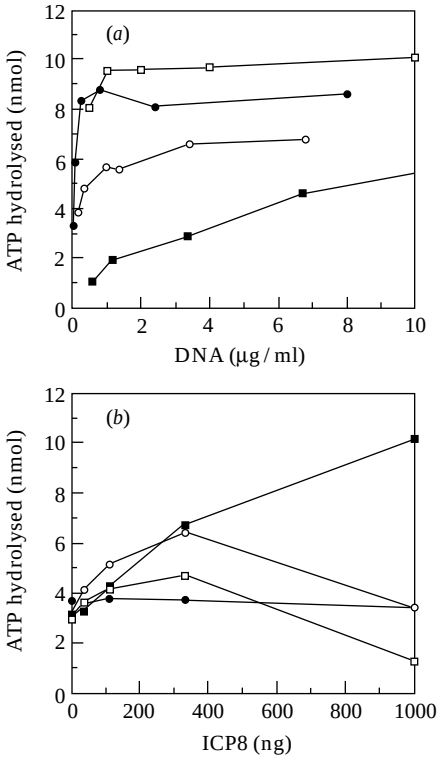

Fig. 5

Fig. 4. Effect of SSBs on UL5/52 and UL5/8/52 ATPase activity. The ATPase activities of (*a*) 1·7 pmol UL5/52 or (*b*) 0·8 pmol UL5/8/52 were measured as described in Methods in the presence of various amounts of SSBs. The results are presented as percentage ATPase activity in the absence of SSB vs the molar ratio of nucleotide to SSB using M_r values of 130000 for ICP8 (○), 75600 for the tetramer of *E. coli* SSB (●) and 33500 for T4 gp32 (□).

Fig. 5. Effect of ICP8 on ATPase activity of UL5/8/52 on various DNAs. ATPase activity was measured at various DNA concentrations (*a*) and at various ICP8 concentrations (*b*). The DNAs used were ϕX174 (○), poly(dT) (●), poly(dA) (■) and poly(dC) (□). In (*a*) 0·8 pmol amounts of UL5/8/52 were used. In (*b*), the amounts of UL5/8/52 was adjusted to give approximately the same ATPase activity in the absence of ICP8 and were 0·8 pmol for ϕX174 (○), 0·4 pmol for poly(dT) (●), 1·6 pmol for poly(dA) (■) and 0·4 pmol for poly(dC) (□).

holoenzyme were inhibited as the DNA became coated with SSBs (Fig. 4). The *E. coli* SSB may be more inhibitory because it binds to a larger region of DNA [33–65 nucleotides per tetramer (Lohman & Overman, 1985)] than either ICP8 [12 nucleotides per monomer (O'Donnell *et al.*, 1987) or T4 gene 32 protein [7–10 nucleotides per monomer (Anderson & Coleman, 1975; Alberts & Frey, 1970; Jensen *et al.*, 1976)]. ICP8, on the other hand, selectively stimulated the UL5/8/52 ATPase activity when present at less than saturating levels with maximal stimulation occurring at one ICP8 per 30–100 nucleotides (Fig. 4*b*). The effect of ICP8 on UL5/8/52 is therefore not limited to its primase activity since it also modulates the ATPase activity with the same specificity and requirement for UL8.

The ability of ICP8 to stimulate UL5/8/52 ATPase activity on various synthetic homopolymer DNAs was evaluated in order to determine if the stimulation was due to the removal of secondary structure from DNA. As shown in Fig. 5(*a*), the synthetic homopolymers differed in their ability to act as effectors for UL5/8/52 with poly(dT) being most effective and poly(dA) being least effective. For this reason, the amount of UL5/8/52 was adjusted for each effector DNA so that the ATPase activities were similar in the absence of ICP8. Under these conditions, ICP8 was able to stimulate UL5/8/52 ATPase activity when ϕX174 and poly(dA) were used as effectors (Fig. 5*b*) indicating that the mechanism of stimulation was not related to removal of secondary structure. Under these same conditions, ICP8 had no effect on poly(dT) and was slightly stimulatory for poly(dC) (Fig. 5*b*). There appeared to be an inverse correlation between the ability of UL5/8/52 to utilize various polynucleotides as effector DNAs and the ability to be stimulated on these polynucleotides by ICP8. The ATPase activity of UL5/8/52 was apparently already at a maximal level on poly(dT) and was not increased further by ICP8. On the other hand, the UL5/8/52 ATPase activity was sub-optimal on poly(dA) and could be increased through its interaction with ICP8. The fact that ICP8 stimulated the ATPase activity of UL5/8/52 on homopolymer DNA indicates that the mechanism of stimulation is not removal of secondary structure.

Effect of ICP8 on UL5/8/52 complex kinetics

It is feasible that ICP8 acts by binding to DNA and facilitating the binding of UL5/8/52. If so, ICP8 stimulation should be more pronounced at low DNA concentrations and negligible at saturating DNA concentrations. The effect of DNA concentration on the rate of UL5/8/52 ATPase activity was therefore determined either in the presence or absence of ICP8 using the two polynucleotides where stimulation was observed. For these experiments the ratio of ICP8 to DNA was maintained at one ICP8 monomer per 150 nucleotides. As can be seen in Fig. 6, the DNA-dependent ATPase activity of UL5/8/52 was stimulated by ICP8 at all concentrations of ϕX174 DNA (Fig. 6*a*) or poly(dA) (Fig. 6*b*). Replots of the data in Fig. 6 as Lineweaver–Burk plots yielded the K_m and k_{cat} values presented in Table 1. For UL5/8/52, ICP8 did not decrease the K_m value for either DNA but instead increased it. This may have been due to ICP8 coating the single-stranded DNA and decreasing the concentration of DNA available for binding to and stimulating UL5/8/52. The k_{cat} of UL5/8/52 was increased 2-fold in the presence of ICP8. These results suggested that the mechanism of ICP8 stimulation was not due to facilitating binding of the helicase–primase complex to DNA but was due to an increase in the enzymatic activity of UL5/8/52.

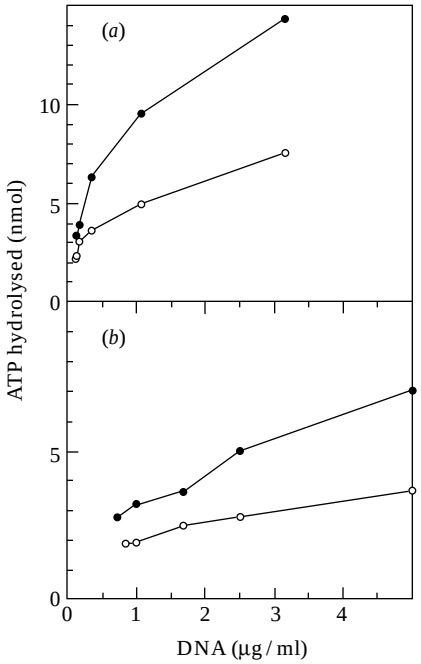

Fig. 6. Effect of ICP8 at various DNA concentrations. ATPase activity was determined at various concentrations (μg/ml) of ϕX174 (*a*) or poly(dA) effector DNA (*b*) with 0·8 pmol amounts of UL5/8/52. Reactions were performed in the absence (○) or presence (●) of ICP8. The ratio of ICP8 to DNA was maintained at one ICP8 monomer per 150 nucleotides.

Table 1. Effect of ICP8 on helicase–primase DNA kinetic parameters

The data in Fig. 6 were fitted to Lineweaver–Burk plots to yield K_m and k_{cat} values.

Effector DNA	ICP8	k_{cat} (s^{-1})*	*K*m (mg/ml)
ϕX174	–	2·2	0·23
	+	4·4	0·38
Poly(dA)	–	1·4	1·1
	+	2·5	1·3

Discussion

The results presented here show that ICP8, the HSV single-stranded DNA-binding protein, stimulated its cognate DNA polymerase and primase activities. Stimulation of the HSV Pol/UL42 activity is non-specific since heterologous SSBs substituted for ICP8. The removal of pause sites by the SSBs, perhaps by melting of secondary structure on the template, suggests that no direct interaction between Pol/UL42 and the SSB is required for this stimulation. Stimulation in the coupled HSV primase–polymerase assay system was specific for ICP8 since only ICP8, but not heterologous SSBs, was stimulatory. The stimulation of UL5/8/52 but not of UL5/52 also suggests that there is an interaction between ICP8 and UL5/52 that is mediated, either directly or indirectly, by UL8.

The stimulation of UL5/8/52 by ICP8 was first observed in the coupled primase–polymerase assay system and was determined to be due to an increase in primer synthesis. The predominant size species of primase product is increased with ICP8 (Tenney *et al.*, 1995; this report, Fig. 3). Additionally, other sized primers are also increased with ICP8, sometimes to a greater extent than is the predominant product (Tenney *et al.*, 1995; this report, Fig. 3). Whether or not this indicates altered primase template initiation specificity will require further investigation. ICP8 was also found to stimulate the DNA-dependent ATPase activity of UL5/8/52. The stimulation of the ATPase activity, like the stimulation of the primase activity, was specific for ICP8 and required the UL8 subunit. Stimulation was observed when ϕX174 DNA or poly(dA) were used as effector DNAs but not when poly(dT), poly(dC) or a 50-mer oligonucleotide (oligo 98) were used as effectors. The inability of UL5/8/52 to be stimulated by ICP8 seemed to correlate with the effectiveness of the DNA ligands in promoting DNA-dependent ATPase activities. Since the effectiveness of the ligands correlated with the ability to bind UL5/8/52 as determined by competition in filter-binding assays (data not shown), a possible mechanism for ICP8 stimulation is that ICP8 bound to DNA promotes the binding of UL5/8/52 to DNA through an interaction with UL8. This is similar to the model proposed for the stimulation of bacteriophage T7 helicase–primase by the T7 SSB (Nakai & Richardson, 1988). However, one prediction of this model is that at saturating DNA concentrations, the k_{cat} for ATP hydrolysis would not be affected by the presence of ICP8. Since ICP8 increases the k_{cat}, ICP8 stimulation occurs through another mechanism. Presumably, a rate-limiting step is facilitated by the interaction of ICP8 with UL8. This rate-limiting step could be any one of the steps involved in helicase activity such as DNA binding, ATP hydrolysis, release of ADP or dissociation from DNA and remains to be determined.

During the drafting of this report, Le Gac *et al.* (1996) published a similar examination of the effect of ICP8 on the HSV helicase–primase. Their work extends ours in showing stimulation of helicase activity. The stimulation was also specific and demonstrated a functional interaction between ICP8 and UL8. However, they reported inhibition of ATPase activity by ICP8, in contrast to our results. This discrepancy is explained by the amounts of ICP8 used: ICP8 stimulation of ATPase is characterized by a relatively narrow concentration optimum and the amounts used by Le Gac *et al.* lie outside the optimal range (this report, Fig. 4). Le Gac *et al.* (1996) also showed inhibition of coupled primase–Sequenase activity by ICP8. We find that ICP8 shows up to a 3-fold stimulation in the coupled primase–HSV polymerase assay and also in the direct primer synthesis assay (Figs 2 and 3, this report; Tenney *et al.*, 1995). While we cannot explain this latter inconsistency, perhaps it originates in differences in protein expression, preparation or polymerase activity.

DNA helicases in several prokaryotic systems are associated with primases, i.e. DnaB with DnaG for *E. coli*, gp41 with gp61 for bacteriophage T4 and the 56 and 66 kDa forms of gp4 for bacteriophage T7 (reviewed by Kornberg & Baker, 1992). The helicase components have been proposed to act as mobile promoters for the primases as they translocate along DNA. Translocation by the helicase is expected to be processive in order to promote efficient DNA replication. The processive nature of bacteriophage T4 gp41 can be seen in the greater extent of GTP hydrolysis effected by very long or by circular DNA (Liu & Alberts, 1981). However, the ATPase activity of the herpes UL5/8/52 appears to be non-processive since small oligonucleotides can very efficiently stimulate DNA-dependent ATPase activity (Tenney *et al.*, 1995, and unpublished data). If the helicase component of UL5/8/52, like the helicase component in the other systems, acts as a mobile promoter, then the ATPase activity results suggest that it may do so by hopping rather than walking along the DNA. In this regard, it would be of interest to determine if the helicase activity of UL5/8/52 is processive or distributive and whether ICP8 can specifically influence helicase functionality.

The finding that the HSV helicase–primase is specifically stimulated by its cognate SSB is not unique to HSV. In the T7 system, primer synthesis and lagging strand DNA replication are specifically stimulated by the T7 SSB and not *E. coli* SSB (Mendelman & Richardson, 1991; Nakai & Richardson, 1988). Furthermore, the T7 primase was stimulated by T7 SSB on an M13 virion DNA template but not on oligomer templates (Mendelman & Richardson, 1991), exactly as we have shown for HSV (Tenney *et al.*, 1995). A recent report supports the specificity of interaction: T7 SSB and primase promote homologous DNA strand exchange while T4 primase and T7 SSB cannot (Kong & Richardson, 1996). The mechanism of these specific interactions is thought to be a physical association as T7 SSB enables the T7 primase to bind to DNA (Nakai & Richardson, 1988). In contrast to the T7 and HSV systems, the T4 SSB specificity is extended to interactions with the T4 polymerase: the T4 SSB (gp32) stimulates polymerase activity while other SSBs do not (Huberman *et al.*, 1971).

In addition to its stimulation of Pol/UL42 activity, ICP8

has recently been shown to stimulate the helicase activity of the HSV UL9 protein *in vitro* (Boehmer *et al.*, 1993; Fierer & Challberg, 1992) and *in vivo* (Boehmer *et al.*, 1994). These two proteins form a tight physical interaction (Boehmer & Lehman, 1993; Boehmer *et al.*, 1994). With this report we can conclude that the primary enzymes involved in HSV DNA replication (polymerase complex, helicase–primase complex, origin binding/unwinding protein) are all specifically affected by the HSV SSB, ICP8. Further tests of the effect of ICP8 on the dynamic functions of these proteins can now be examined in *in vitro* systems involving multiple proteins and activities.

We thank I. Robert Lehman for recombinant baculoviruses expressing HSV helicase–primase and UL30 proteins. We appreciate the critical comments on the manuscript by Amy Sheaffer.

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Received 14 October 1996; Accepted 17 December 1996