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The UL8 Subunit of the Heterotrimeric Herpes Simplex Virus Type 1 Helicase-Primase Is Required for the Unwinding of Single Strand DNA-binding Protein (ICP8)-coated DNA Substrates*

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The Herpes simplex virus type 1 primosome consists of three subunits that are the products of the *UL5*, *UL8*, and *UL52* genes. The heterotrimeric enzyme has DNA-dependent ATPase, helicase, and primase activities. Earlier studies show that a subassembly consisting of the *UL5* and *UL52* gene products was indistinguishable from the heterotrimeric enzyme in its helicase and primase activities. We demonstrate here that the UL8 protein is required for the helicase activity of the UL5/52 subassembly on long duplex DNA substrates (>30 nucleotides) with a single-stranded DNA loading site fully coated with the virus-encoded single strand DNA binding protein, ICP8. The *Escherichia coli* single strand DNA binding protein cannot substitute for ICP8, suggesting a specific physical interaction between ICP8 and the UL8 protein. Surface plasmon resonance measurements demonstrated an interaction between ICP8 and the UL5/52/8 heterotrimer but not with the UL5/52 subassembly or the UL8 protein alone. At a subsaturating level of ICP8, the UL5/52 subassembly does show helicase activity, suggesting that the subassembly can bind to single-stranded DNA but not to ICP8-coated DNA.

Herpes simplex virus type 1 (HSV-1)[1] encodes seven proteins that are essential for the replication of its genome (1). These include an origin binding protein (2, 3), a single strand DNA binding protein (4), a DNA polymerase (5) with its associated processivity factor (6–8), and a heterotrimeric primosome. The primosome encoded by HSV-1 is composed of the products of the *UL5*, *UL52*, and *UL8* genes and has DNA-dependent ATPase, DNA helicase, and DNA primase activities (9–12). Although the *UL8* gene product is essential for HSV-1 DNA replication *in vivo*, a subassembly consisting of only the 99-kDa *UL5* and 114-kDa *UL52* gene products was found to be indistinguishable in its DNA-dependent ATPase, DNA helicase, and DNA primase activities from the heterotrimeric enzyme (13, 14). It was subsequently observed that the 80-kDa UL8 protein, which lacks enzymatic and DNA binding activities, can stimulate primer synthesis (15–16). It can also enhance the utilization of primers synthesized by the UL5/52 heterodimer (17). More recently, it was found that the UL8 protein can stimulate the DNA-dependent ATPase, helicase, and primase activities of the UL5/52 heterodimer in the presence of the HSV-1-encoded single strand DNA-binding protein, ICP8 (18). In the present study we have found that with duplex DNA substrates >30 nucleotides in length containing a single-stranded loading site fully coated with ICP8, helicase activity shows an almost complete dependence on the UL8 subunit. However, at a narrow range of subsaturating levels of ICP8, unwinding of the helicase substrate can be promoted by the UL5/52 subassembly alone. The helicase activity of the HSV-1-encoded primosome therefore appears to be modulated by ICP8.

MATERIALS AND METHODS

Cells and Viruses—Spodoptera frugiperda (Sf9 and Sf21) cells were grown at 27 °C in Sf-900 11 SFM medium (Life Technologies, Inc.) on a gyratory shaker rotating at 150 rpm.

Stocks of *Autographa californica* nuclear polyhedrosis virus recombinant for the *UL8*, *UL5*, *UL52*, and *UL29* genes were prepared by infecting 500 ml of SF9 cells grown to 1×10^6 cells/ml at 0.1 plaque-forming unit/cell. The medium was supplemented with 5% fetal calf serum (Life Technologies, Inc.) just before infection. After incubation for 3 days, the cells were pelleted by centrifugation ($1500 \times g$, 10 min) at room temperature, and the virus in the supernatant fluid was either used directly or frozen at −80 °C. The final titers were approximately 10^7–10^8 plaque forming units/ml.

Preparation of Infected Cell Extracts—Five hundred milliliters of Sf21 cells were grown to a density of 2×10^6 cells/ml in medium supplemented with 1% fetal calf serum. The cells were infected with 5–10 plaque forming units/cell recombinant baculovirus, grown for 48 h, and pelleted by centrifugation ($1500 \times g$, 10 min) at 4 °C. After washing with 500 ml of ice-cold phosphate-buffered saline (Life Technologies, Inc.), the cells were collected by centrifugation ($1500 \times g$, 10 min) at 4 °C. The pellet was frozen in liquid nitrogen and kept at −80 °C. To lyse the cells, the frozen cell pellet was resuspended in 5 volumes of ice-cold lysis buffer containing 20 mM Hepes (pH 7.6), 1.0 mM dithiothreitol, 10 mM sodium bisulfite (pH 7.8), 5 μg/ml leupeptin, 5 μg/ml pepstatin A, 0.5 mM phenylmethylsulfonic acid, 1.0 mM EDTA (pH 8.0), and 1 mM EGTA (pH 8.0). The resuspended cells were incubated for 20 min on ice, transferred to a Dounce homogenizer, and disrupted using 15 strokes of a tight-fitting pestle. For the UL5/52 and UL5/52/8 proteins, nuclei were removed from the extract by centrifugation ($500 \times g$, 10 min) at 4 °C. The cytosolic fraction was used directly or frozen in liquid nitrogen and stored at −80 °C. In the case of ICP8, NaCl was added to the suspension of nuclei to a final concentration of 1.2 M, and the mixture was rotated for 45 min at 4 °C. The extract was cleared by centrifugation at 55,000 rpm for 60 min using a Beckman 70.1 Ti rotor.

Enzyme Purification—The UL5/52/8 and UL5/52 proteins were purified as described previously (19), with the following modifications. Heparin-agarose was replaced with a prepacked 10-ml heparin Hi-trap column (Pharmacia Biotech Inc.), and the Superose 12 column was replaced with a prepacked Sephadex 200 (16/60) column (Pharmacia). The yields of UL5/52/8 heterotrimer and UL5/52 heterodimer were approximately 5 mg each. The purity of both complexes estimated by

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[1] The abbreviations used are: HSV-1, herpes simplex virus type 1; SSB, single-stranded DNA-binding protein; bp, base pair(s).

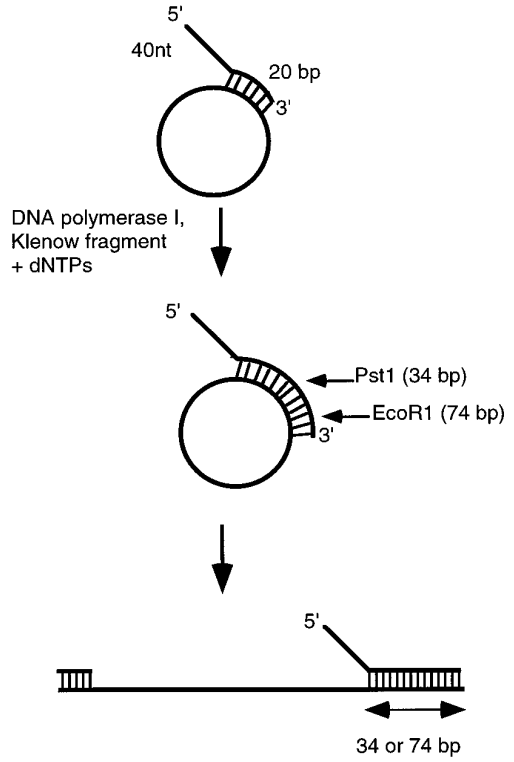

FIG. 1. **Formation of helicase substrates.** The helicase substrates were generated by annealing a 60-bp oligonucleotide labeled with ^{32}P at its 5′ terminus with T4 polynucleotide kinase and [α-^{32}P]ATP to single-stranded M13 mp18 DNA. The 20-bp duplex region was extended by incubation with the Klenow fragment of DNA polymerase I in the presence of Mg^{2+} and the four deoxynucleoside triphosphates. Subsequent cleavage with *Pst*I or *Eco*RI created substrates containing duplex regions with the lengths indicated. *nt*, nucleotides.

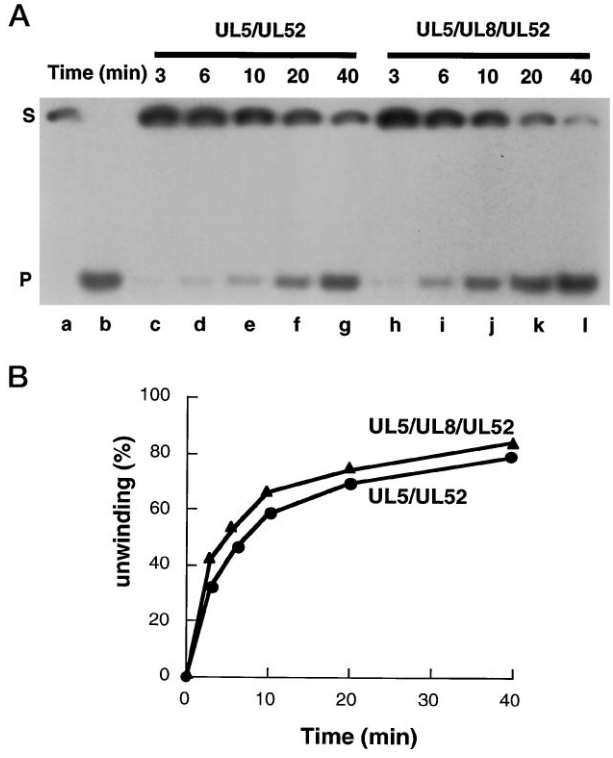

FIG. 2. **The helicase activity of the UL5/52 subassembly is indistinguishable from that of the UL5/52/8 heterotrimer on short duplex DNA substrates in the absence of ICP8.** Reaction mixtures were prepared as described under "Materials and Methods" except that the volume was 70 μl. The substrate (105 fmol) contained a 20-bp double-stranded region and a 40-nucleotide 5′ tail. The UL5/52 subassembly (525 fmol) and the UL5/52/8 heterotrimer (525 fmol) were added. Samples (10 μl) were removed at the times indicated, and 2 μl of stop solution was added. *A*, the products were subjected to nondenaturing polyacrylamide gel electrophoresis as described under "Materials and Methods." *S*, substrate; *P*, product. *Lane a*, untreated substrate; *lane b*, substrate heated to 100 °C before electrophoresis. *B*, the gel was scanned with a PhosphorImager (Molecular Dynamics), and the fractional amounts of base-paired substrate and single-stranded product were determined.

SDS-polyacrylamide gel electrophoresis followed by Coomasie Blue staining was at least 95%.

The UL8 protein was purified as described previously (14), with the following modifications. Two prepacked 5-ml heparin Hi-trap columns coupled in series were used in place of the heparin-agarose column, and Superose 12 was replaced by a Sephadex 200 (16/60) column. The yield of UL8 protein was approximately 6 mg. It was at least 95% pure. ICP8 was purified as described previously (20). All proteins were frozen in liquid nitrogen and stored at −80 °C. *Escherichia coli* SSB was obtained from Amersham Life Sciences, Inc.

Helicase Substrates—A 60-base oligonucleotide (5′-ACATGATAAGATACATGGATGAGTTTGGACAAACCACAACGTAAAACGACGGCCAGTGCC-3′) was labeled with ^{32}P at its 5′ terminus with T4 polynucleotide kinase (Stratagene) and annealed to M13 mp18 single-stranded DNA (Biolab) to generate a 20-bp double-stranded region with a 40-nucleotide 5′ tail. To extend the duplex, it was incubated with the Klenow fragment of DNA polymerase I in the presence of the four deoxynucleoside triphosphates and Mg^{2+} (21). The DNA product was subsequently cleaved with the *Pst*I or *Eco*RI restriction enzymes to yield a double-stranded region 33- or 74-bp in length, respectively. The DNAs were purified by gel filtration through a 1-ml Bio-Gel A-5 m column (Bio-Rad) that had been equilibrated in buffer containing 20 mM Tris-HCl (pH 7.6), 100 mM NaCl, and 0.1 mM EDTA.

Helicase Assay—The reaction mixture (10 μl) contained 15 fmol of DNA substrate, 20 mM Tris-HCl (pH 7.6), 10% glycerol, 5 mM dithiothreitol, 4.5 mM $MgCl_2$, 3 mM ATP, 100 μg/ml bovine serum albumin, 40 mM NaCl, and the indicated amounts of ICP8 and the UL5/52/8 or UL5/52 proteins. Incubation was at 32 °C for the times indicated and stopped by the addition of 2 μl of stop solution (90 mM EDTA (pH 8.0), 6% SDS, 30% glycerol, 0.25% bromphenol, 0.25% xylene cyanol). The products were separated by electrophoresis through an 8% nondenaturing polyacrylamide gel, dried onto DE81 (Whatman), and autoradiographed overnight at −80 °C with an intensifying screen. An extra step was added in the experiments involving *E. coli* SSB. 10 μl of 400 μg/ml proteinase K and 2% SDS were added, and the mixture was incubated for an additional 10 min at 32 °C before 4 μl of stop solution was added.

Surface Plasmon Resonance—Measurements of the interaction of the UL8 protein, the UL5/52 subassembly, and the UL5/52/8 heterotrimer with ICP8 were performed using the BIAcore Biosensor (Biacore AB Pharmacia). ICP8 was immobilized on a CM5 research grade sensor chip at 140 μg/ml in 100 mM sodium acetate (pH 6.0) with the use of an amine coupling kit (Biacore AB) (22, 23). A blank surface was prepared by injecting 100 mM sodium acetate (pH 6.0) onto a CM5 research grade sensor chip. Measurements were performed at room temperature in 20 mM Hepes (pH 7.6), 4.5 mM $MgCl_2$, 50 mM sodium chloride, 5% glycerol, 1 mM dithiothreitol, and 0.005% surfactant P-20 with a flow rate of 15 μl/min. All surfaces were washed with 20 mM Hepes (pH 7.6), 4.5 mM $MgCl_2$, 1 M sodium chloride, 10% glycerol, 1 mM dithiothreitol, and 0.005% surfactant P-20 to remove noncovalently bound proteins.

RESULTS

The UL8 Protein Is Not Required for Helicase Activity on Short DNA Substrates with a Single Strand Loading Site—A ^{32}P-labeled 60-base oligonucleotide was annealed to the complementary region of M13 mp18 single-stranded DNA to form a helicase substrate with a 20-bp double-stranded region and a 40-nucleotide-long 5′ single-stranded tail (Fig. 1). As shown in Fig. 2, *A* and *B*, the helicase activity of the UL5/52 subassembly with this substrate was almost indistinguishable from that of the heterotrimeric enzyme. This result is in agreement with earlier reports (14, 15). The effect of ICP8 on the helicase action of the UL5/52/8 and UL5/52 proteins could not be tested because of the helix-destabilizing activity of ICP8 on short duplexes under these conditions (24).

The UL8 Protein Is Required for Helicase Activity on Long

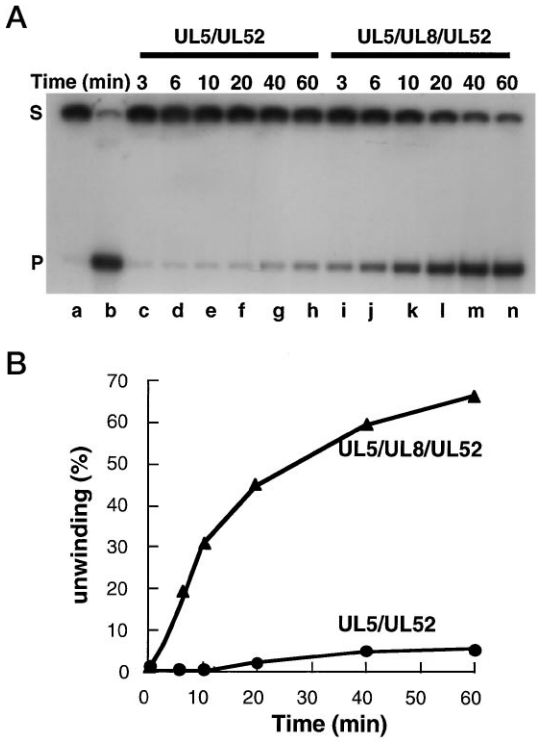

FIG. 3. **Effect of ICP8 on the helicase activity of the UL5/52 subassembly and UL5/52/8 heterotrimer acting on long duplex DNA substrates.** Reaction mixtures were prepared as described under "Materials and Methods" except that the volume was 70 μl. The substrate (105 fmol) contained a 74-bp duplex region and a 40-nucleotide 5′ tail, and ICP8 (70 pmol), and 525 fmol each of the UL5/52 subassembly and the UL5/52/8 heterotrimer were added. Samples (10 μl) were removed at the times indicated, and 2 μl of stop solution was added. *A*, the products were analyzed by nondenaturing polyacrylamide gel electrophoresis as described in Fig. 2. *Lane a*, untreated substrate. *Lane b*, substrate heated to 100 °C before electrophoresis. *S*, substrate; *P*, product. *B,* the gel was scanned with a PhosphorImager, and the fractional amounts of base-paired substrate and single-stranded DNA product were determined.

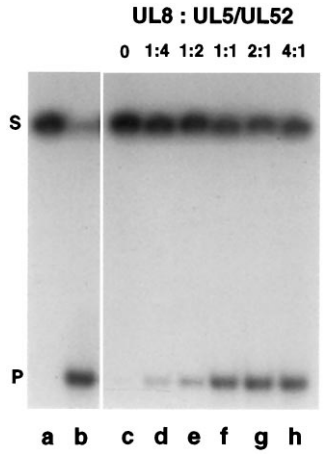

FIG. 4. **Maximal stimulation of helicase activity by ICP8 occurs at a 1:1 ratio of UL8 protein to UL5/52 subassembly.** Reaction mixtures were prepared as described under "Materials and Methods." Ten pmol of ICP8, 75 fmol of the UL5/52 subassembly, and 75 fmol of substrate consisting of a 74-bp duplex region with a 40-nucleotide 5′ tail were added together with increasing amounts of UL8 protein. The molar ratio of UL8 protein to the UL5/52 subassembly is indicated (*lanes c–h*). After 30 min, the reactions were stopped, and the products were analyzed by nondenaturing polyacrylamide gel electrophoresis as described in Fig. 2. *Lane a*, untreated substrate; *lane b*, substrate heated to 100 °C before electrophoresis. *S*, substrate; *P*, product.

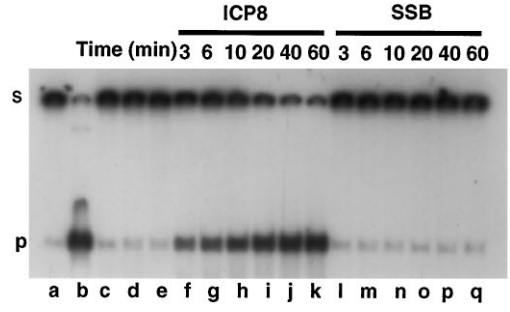

FIG. 5. **Stimulation of the helicase activity of UL5/52/8 heterotrimer is ICP8-specific.** Reaction mixtures (70 μl) were prepared as described under "Materials and Methods," except that ICP8 (70 pmol) or *E. coli* SSB (70 pmol) was incubated with the UL5/52/8 heterotrimer (525 fmol). The substrate (105 fmol) contained a 74-bp duplex region with a 40-nucleotide 5′ tail. Samples (10 μl) were removed at the times indicated and analyzed by nondenaturing polyacrylamide gel electrophoresis as described in Fig. 2. *Lane a*, untreated substrate; *lane b,* substrate heated to 100 °C before electrophoresis; *lane c*, UL5/52/8 heterotrimer incubated for 60 min without ICP8 or *E. coli* SSB. *Lanes d* and *e*, ICP8 and SSB, respectively, incubated for 60 min in the absence of the UL5/52/8 heterotrimer. *S*, substrate; *P*, product.

Duplex DNA Substrates with an ICP8-coated Single Strand Loading Site—We reasoned that the UL8 protein might be required to enhance the processivity of the helicase-primase, an effect that would be missed with short DNA duplexes. We therefore increased the length of the double-stranded region by DNA polymerase action followed by cleavage with the appropriate restriction enzymes. The substrates produced in this way contained duplex stretches of 33- and 74-bp in addition to the 40-bp single strand loading site (Fig. 1).

With the 74-bp duplex substrate, essentially no unwinding by the UL5/52 heterodimer could be detected either in the presence or absence of the UL8 protein (<1%). However, when ICP8 was added, there was a significant difference in the response of the UL5/52 and UL5/52/8 proteins. In the absence of the UL8 subunit, ICP8 had only a very small stimulatory effect; in its presence, substantial unwinding was observed (Fig. 3, *A* and *B*). Essentially the same result was obtained with the substrate containing a 34-bp duplex region (data not shown). We therefore conclude that ICP8 has a strong stimulatory effect on the helicase activity of the HSV-1 helicase-primase and that this stimulation is dependent upon the UL8 protein.

Stoichiometric Amounts of UL8 Protein Are Required for Maximum Helicase Activity—To determine the amount of UL8 protein required for optimal helicase activity, increasing amounts in the UL8 protein were added to the UL5/52 subassembly, and helicase activity was determined with the substrate containing a 74-bp DNA duplex and a 40-nucleotide single strand loading site coated with ICP8. As shown in Fig. 4, helicase activity reached a plateau when the UL8 protein was present at a ratio of about 1:1 with the UL5/UL52 subassembly. This finding is consistent with the 1:1:1 stochiometry that is found in the helicase-primase isolated from HSV-1-infected cells (10, 12).

Stimulation of Helicase-Primase Is ICP8-specific—To determine whether stimulation of the helicase activity of the UL5/52/8 heterotrimer by ICP8 is specific, the heterologous *E. coli* SSB was substituted for ICP8. As shown in Fig. 5, there was no stimulation of helicase activity by the *E. coli* SSB under conditions where ICP8 produced a strong stimulation. The T4 phage SSB, gene 32 protein, was similarly ineffective (data not shown). The specific stimulation of helicase activity by ICP8 suggested that there is physical interaction between ICP8 and the helicase-primase. However, attempts to detect such an

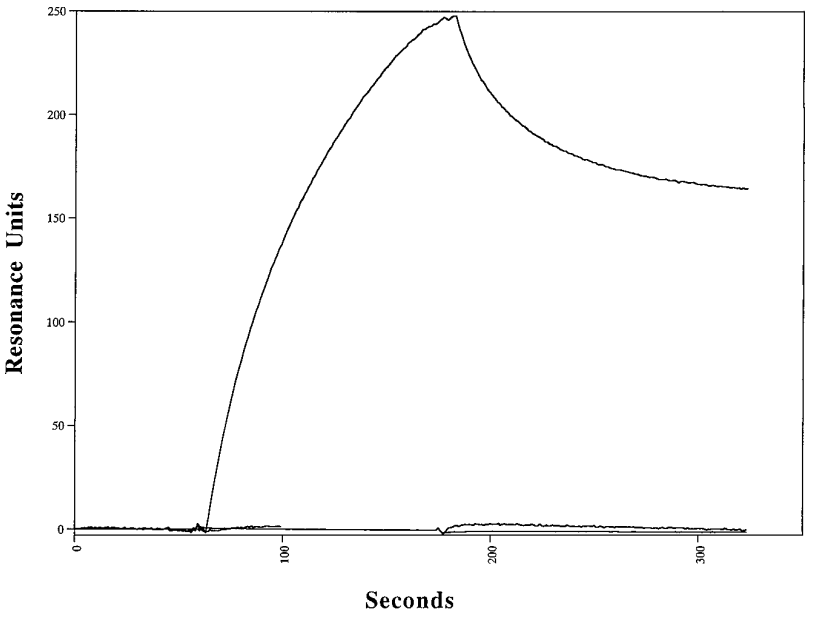

FIG. 6. **Interaction of the UL5/52 heterodimer requires UL8 protein to interact with ICP8.** Surface plasmon resonance measurements were performed with the BIAcore Biosensor as described under "Materials and Methods." Sensograms of 12.5 μg/ml UL5/52 subassembly (*bottom curve*), 5 μg/ml UL8 protein (*middle curve*), and 17 μg/ml UL5/52/8 holoenzyme (*top curve*) were injected onto the surface of immobilized ICP8.

interaction by specific retention of either the UL8 protein or the UL5/52/8 heterotrimer on an ICP8-agarose column or by co-immunoprecipitation with antibody directed against ICP8 were unsuccessful.[2]

Demonstration of the Interaction between ICP8 and UL5/52/8 Holoenzyme by Surface Plasmon Resonance—Surface plasmon resonance detects the change in refractive index near a surface bearing one adsorbed protein as a result of the interaction with a second protein. The change in refractive index, in resonance units, recorded as a function of time after the addition of the second protein or following removal of this protein can be used to determine the rates of association and dissociation respectively. These rates are related through simple exponentials to the fundamental rate constant k_a and k_d for the binding reaction,

$$A + B \underset{k_d}{\overset{k_a}{\rightleftharpoons}} AB \qquad \text{(Eq. 1)}$$

with $k_a/k_d = K_A$, the equilibrium association constant (25).

In these experiments, ICP8 was immobilized to the dextran surface of a BIAcore sensor chip, and its interaction with the UL5/52/8 holoenzyme, UL5/52 subassembly, and the UL8 protein was measured. As shown in Fig. 6, the UL5/52 subassembly and the UL8 protein failed to interact with the ICP8 surface. In contrast, the UL5/52/8 holoenzyme did interact significantly. The apparent association rate constant (k_a) was 2.8×10^5 s^{-1} M^{-1} (S.D., 8.5×10^4; $n = 4$). The apparent dissociation rate constant (k_d) was 2.5×10^{-3} s^{-1} (S.D., 9.8×10^{-4}; $n = 4$). We assume that a 1:1 complex is formed between ICP8 and the UL5/UL52/UL8 heterotrimer. A plot of $\ln(R_0/R)$ *versus* time after the start of dissociation, where R_0 is the response at the start of dissociation and R is the response at time t after dissociation is linear, indicating that dissociation is first order (data not shown).

Subsaturating Levels of ICP8 Can Stimulate the Helicase Activity of the UL5/52 Subassembly—The experiments described this far were all performed at excess ICP8 to ensure that free ICP8 would be available to bind the single-stranded DNA generated during the helicase-promoted unwinding of the substrate (26, 27). Under these conditions, the UL5/52 heterodimer was inactive. There was, however, a narrow range of subsaturating ICP8 concentrations at which unwinding of the substrate could be observed in the absence of the UL8 protein (Fig. 7, *A* and *C*). As the level of ICP8 was increased, the stimulatory effect was suppressed. In contrast, increasing amounts of ICP8, in the presence of the UL8 protein, produced no such inhibition (Fig. 7, *B* and *C*). In a control experiment using *E. coli* SSB, no stimulation of helicase activity was observed at any of the concentrations tested. It therefore appears that low concentrations of ICP8 can have a specific stimulatory effect on the helicase activity of the helicase-primase even in the absence of the UL8 protein.

DISCUSSION

The primosome encoded by HSV-1 is a heterotrimer consisting of the products of the *UL5*, *UL8*, and *UL52* genes (9–12). Helicase activity is associated with the UL5 subunit (17, 28), and primase activity is associated with the UL52 subunit (29). The specific function of the UL8 subunit has remained obscure. In the studies reported here, we have found that the UL8 subunit is not needed for helicase action on short, 20-nucleotide duplex DNA substrates in the absence of ICP8 but is required for longer, 30–74-bp duplex DNA substrates with a single-stranded loading site fully coated with the HSV-1 single strand DNA binding protein, ICP8. A plausible inference from these findings is that the UL8 subunit of the primosome interacts with ICP8-coated single-stranded DNA to facilitate binding of the UL5/52 heterodimer. The reaction is specific for ICP8, and a heterologous single strand DNA-binding protein (*E. coli* SSB) was ineffective, suggesting a physical interaction of the primosome with ICP8.

By using surface plasmon resonance we did, in fact, observe that the UL5/8/52 holoenzyme, but neither the UL5/52 subassembly nor the UL8 subunit alone, was able to bind to ICP8. The estimated k_d of 2.5×10^{-3} s^{-1} confirmed that the interaction is specific and occurs with a relatively high affinity. Attempts to measure the interaction of the UL5/52/8 holoenzyme, the UL5/52 subassembly, or the UL8 protein with ICP8 bound to single-stranded DNA were complicated by the rapid association and dissociation of ICP8 with and from the single-stranded DNA.[3]

[2] M. Falkenberg, P. Elias, and I. R. Lehman, unpublished data.

[3] D. Bushnell, M. Falkenberg, P. Elias, and I. R. Lehman, unpublished data.

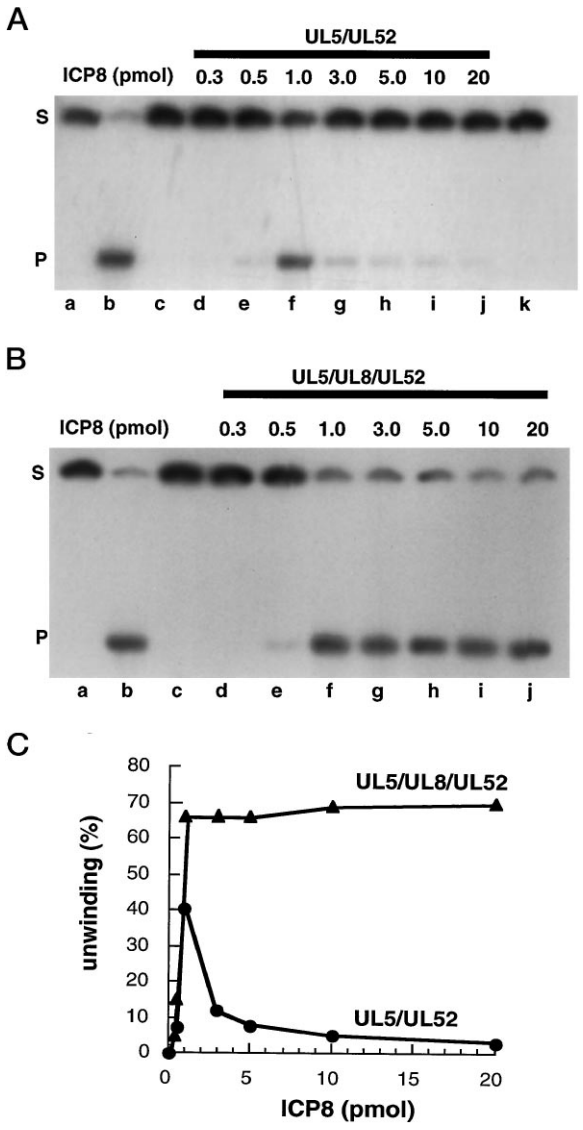

FIG. 7. **Low concentrations of ICP8 can stimulate the helicase activity of UL5/52 subassembly in the absence of UL8 protein.** Reaction mixtures were prepared as described under "Materials and Methods." The UL5/52 subassembly (*A*) and the UL5/52/8 heterotrimer (*B*) were present at 75 fmol, and increasing amounts of ICP8 were added as indicated. The substrate (105 fmol) contained a 74-bp duplex region with a 40-nucleotide 5′ tail. Incubation was for 60 min. *A*, *lane a,* untreated substrate; *lane b,* substrate heated to 100 °C before electrophoresis. *Lane c,* the UL5/52 subassembly alone; *lanes d–j*, increasing amounts of ICP8 were added; *lane k*, ICP8 alone. *B*, *lane a*, untreated substrate; *lane b*, substrate heated to 100 °C; *lane c*, UL5/52/8 heterotrimer alone; *lanes d–j*, increasing amounts of ICP8 were added. *S*, substrate; *P*, product. *C*, the gels were scanned with a PhosphorImager, and the fractional amounts of base-paired substrate and single-stranded DNA product were plotted against the ICP8 added. The substrate was fully coated with ICP8 when 7.5 pmol of ICP8 was added (the binding site size for ICP8 is 18–22 nucleotides (24, 31)).

What is the mechanism by which the UL8 protein promotes the binding of the helicase-primase to an ICP8-coated substrate? One possibility is that it permits the displacement of ICP8 from DNA, perhaps by stabilizing a conformation of ICP8 that prevents it from binding to single-stranded DNA. Alternatively, ICP8-coated single-stranded DNA itself may serve as the loading site for the helicase-primase. Since the UL8 protein alone does not bind ICP8, interaction of the UL8 protein with the UL5/52 subassembly either enhances its binding to ICP8, or alternatively, facilitates interaction of the UL5/52 subassembly with ICP8.

The effects of ICP8 on the HSV-1 primosome are reminiscent of the effects of the T4 bacteriophage single strand DNA-binding protein, the gene 32 protein, on the T4 primosome, the product of T4 genes 41 and 61. The gene 32 protein stimulates the helicase activity of the primosome but confines the synthesis of RNA primers to those sites that are used to initiate synthesis of an Okazaki fragment (30). An additional T4-encoded protein, the product of gene 59, is required to assemble the gene 41 protein (the helicase) onto gene 32-coated single-stranded DNA. This reaction is mediated by a specific interaction between the gene 59 and gene 32 proteins (30). Thus, the HSV-1-encoded UL8 protein may be the functional analogue of the T4 gene 59 protein.

A surprising finding was that over a narrow range of subsaturating ICP8 concentrations, the UL5/52 subassembly can unwind long DNA duplexes even in the absence of the UL8 subunit. Possibly the UL5/52 heterodimer can bind to a single-stranded DNA loading site free of ICP8 and promote helicase activity. The appropriate concentration of ICP8 would then be needed to bind the single strands produced by helicase action, preventing them from reannealing. At levels of ICP8 sufficient to fully coat the single-stranded DNA, the UL5/52 subassembly is unable to bind the ICP8-coated single strands, and helicase activity is inhibited.

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