Replacement of Gly$^{815}$ in Helicase Motif V Alters the Single-stranded DNA-dependent ATPase Activity of the Herpes Simplex Virus Type 1 Helicase-Primase*

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Herpes simplex virus type 1 encodes a helicase-primase complex composed of the products of the UL5, UL52, and UL8 genes. A subcomplex consisting of the UL5 and UL52 proteins purified from insect cells also displays ATPase, helicase, and primase activities. UL5 contains six motifs conserved in superfamily I of known and/or putative helicase proteins. Consistent with the ability to hydrolyze ATP, motifs I and II resemble a nucleotide binding site. Although the role of the other four motifs is not known, single amino acid substitutions created in conserved residues in all six motifs abolish the ability of UL5 to support viral DNA replication in vivo (Zhu, L., and Weller, S. K. (1992) J. Virol. 66, 469-479). In one such mutation, a highly conserved glycine in motif V (Gly$^{815}$) is replaced with an alanine. Although the UL5(G815A) protein does not support viral DNA replication in vivo, the purified UL5(G815A)-S2 subcomplex retains primase and helicase activities and supports strand displacement DNA synthesis on a preformed replication fork in the presence of the other HSV-1 replication proteins. The major difference between the wild-type and variant protein is that the UL5(G815A)-S2 subcomplex displays an increased $K_m$ for single-stranded DNA and decreased $K_{cat}$ for single-stranded DNA-dependent ATPase activity. Several hypotheses for the role of motif V in the function of the UL5 helicase in HSV-1 DNA replication are considered. This is the first report of a biochemical analysis of a motif V variant in any member of helicase superfamily I.

Transient unwinding of duplex DNA by a helicase is crucial to many biological processes such as DNA replication, recombination, and repair. Helicases have been isolated and characterized from a variety of both eukaryotic and prokaryotic organisms (reviewed in Refs. 1-3). DNA helicases translocate along single-stranded DNA (ssDNA) to catalyze unwinding of double-stranded DNA with the energy provided by NTP hydrolysis. DNA helicases are also classified as ssDNA-dependent NTPases. DNA helicases can be distinguished by their polarity of migration (5' to 3' or 3' to 5') and their mode of interaction with DNA (distributive or processive). Many DNA helicases have also been shown to have an associated primase activity, including the DnaB-DnaG complex from Escherichia coli, gp4 from bacteriophage T7, and the gp42-61 complex from bacteriophage T4 (reviewed in Ref. 4).

Herpes simplex virus type 1 (HSV-1) encodes seven proteins essential for viral DNA replication. These include a subunit DNA polymerase (UL30-42), an ssDNA binding protein (UL29), an origin-specific DNA binding protein (UL9), and a heterotrimeric complex composed of the products of the UL5, UL52, and UL8 genes (reviewed in Refs. 5-7). The UL5-52-8 complex has been shown to possess ssDNA-dependent ATPase, 5' to 3' DNA helicase, and RNA primase activities (8-11). Although all three subunits are required for activity in vivo (7, 12-14), the UL8 protein is not required for helicase or primase activities in vitro (8, 11). The UL52 protein contains a motif conserved in many primases that when mutated, abolishes primase but not ATPase or helicase activities of the heterotrimeric complex (15, 16). This suggests that UL52 encodes the primase and not the helicase component of the complex.

Protein sequence analysis has revealed that UL5 possesses six motifs conserved among superfamily I of known and/or putative helicase proteins (17, 18). Included in this superfamily are at least 12 proteins from members of the herpesvirus family (putative UL5 homologs), E. coli proteins (UvrD, Rep, RecB, and RecD), a yeast mitochondrial protein (PIF), and a number of positive strand RNA viral proteins (see Fig. 1). Consistent with the ability to hydrolyze ATP, these proteins contain two highly conserved sequence motifs (motifs I and II) that define a nucleotide binding site (19). Replacement of conserved residues in either of these motifs generally leads to a loss of both ATPase and helicase activities in a number of helicases including E. coli proteins UvrB (20) and UvrA (21), the gp4 protein from bacteriophage T7 (22), and the yeast RAD3 protein (23). Mutation of a conserved lysine in motif I of UL5 abolishes both ATPase and helicase activity. Although the functional significance of motifs III, IV, V, and VI are not well understood, the strong conservation of the six motifs suggests that these sequence elements may be important for helicase activity. A number of models have been proposed for the catalytic mechanism of DNA helicases (24, 25); however, the exact mechanism by which duplex DNA is unwound remains unknown.

Previous work in this laboratory has shown that single amino acid substitutions in each of the six helicase motifs abolish the ability of UL5 to complement a UL5 null virus for replication of an HSV-1 origin-containing plasmid in vivo (26).

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1The abbreviations used are: ssDNA, single-stranded DNA; HSV-1, herpes simplex virus type 1; S19, S. frugiperda; DTT, dithiothreitol; kb, kilobase(s); TAPS, 3-[2-hydroxy-1,1-bis(hydroxymethyl)ethylamino]-1-propanesulfonic acid.

2J. Crute, personal communication.
This is consistent with the notion that all six motifs are essential for the function of the UL5 protein in HSV-1 DNA replication. Motif V contains several conserved residues; most members of superfamily I contain the sequence TXQGXXV (27) (Fig. 1B). Another superfamily of helicase proteins (superfamily II) also contains six well conserved motifs (28). Motifs I and II are well conserved between superfamily I and II. Although the sequences of the other four motifs are less well conserved, it has been previously overlooked that many members of superfamily II also contain the invariant Thr, Gly, and Val residues. Replacement of the invariant glycine in the yeast RAD3 helicase (superfamily II) results in an increase in the levels of recombination between short homologous sequences (29). In the E. coli UvrB helicase (superfamily II), replacement of this glycine and other residues in motif V reduces the level of UV-damaged DNA-inducible ATPase activity in vitro (30). The substitution of the invariant glycine at position 815 in HSV-1 UL5 results in the failure to support replication of an HSV-1 origin-containing plasmid in vivo (26). In this report we characterize the biochemical properties of this variant protein in vitro. The recombinant UL5-52 subcomplex containing either the wild-type UL5 or the UL5(G815A) protein expressed in insect cells was purified. We report that the UL5(G815A)-52 subcomplex displays an altered ability to catalyze ssDNA-dependent ATP hydrolysis but retains many of the other activities catalyzed by the wild-type enzyme in vitro.

**EXPERIMENTAL PROCEDURES**

**Reagents**

Supplemented Grace's medium, fetal calf serum, and 10% Pluronic® F-68 were purchased from Life Technologies, Inc. Baculogold™ DNA was obtained from Pharmingen. Penicillin-streptomycin solution and ampicillin were from Sigma. All restriction enzymes were purchased from New England Biolabs. The MonoQ® HR 5/5 anion exchange column and the Superose® 12 HR 10/30 gel filtration column were from Pharmacia Biotech Inc. Radiolabeled nucleotides were purchased from Amersham Corp. Phenylmethylsulfonyl fluoride, leupeptin, and pepstatin were from Sigma. Oligonucleotides were synthesized by National Biosciences, Inc. Long Ranger™ 50% acrylamide was purchased from AT Biochem. Double-stranded DNA cellulose was obtained from Pharmacia. ssDNA cellulose was a kind gift from Dr. Earl F. Baril (Worcest Foundation for Experimental Biology). Purified UL30-42 protein preparations were kind gifts from Drs. Daniel Tenney and Robert Hamatake (Bristol-Myers Squibb, Wallingford, CT) and from Dr. Mark Chalfell (National Institutes of Health, Bethesda, MD). The baculovirus expression plasmid pAcCL29 was a kind gift from Dr. Vivienne F. Murphy (SmithKline Beecham, Surrey, UK).

**Buffers**

Buffer A was 20 mM Na+, 10 mM sodium bisulfite, 0.5 mM MgCl2, 0.5 mM phenylmethylsulfonyl fluoride, 2 μg/ml leupeptin, and 2 μg/ml pepstatin. Buffer B was Buffer A to which 10% (v/v) glycerol and 0.5 mM EDTA were added and from which sodium bisulfite was omitted. All buffers were filtered through a 0.22-μm membrane and degassed before use.

**Cells, Viruses, and DNA**

Sporplota frugiperda (SF9) cells were grown in Grace's insect medium containing 10% fetal calf serum, 0.1 mg/ml streptomycin, and 100 units/ml penicillin. Recombinant Autographa california virus nuclear polyhedrosis baculoviruses AcUL5, AcUL52, AcUL8, AcUL30, AcUL42, and AcUL29 were kind gifts from Dr. Mark Chalfell (National Institutes of Health). Viral stocks were amplified in SF9 cells grown in suspension (supplemented with 0.01% Pluronic® F-68) by infecting at a multiplicity of infection of 0.01 plaque-forming units/cell for 6 days at 27°C. Stocks were titrated by plaque assay using 1 × 106 SF9 cells/35-mm plate for 6 days. Plaques were visualized by staining with 10% neutral red in phosphate-buffered saline. Viral stocks were stored at 4°C protected from light. All plasmid DNAs were propagated in the E. coli strain UT481 in Luria broth containing 100 μg/ml ampicillin.

**Construction of the Baculovirus Recombinant Harboring the UL5 Motif V Mutant Gene**

The plasmid p6UL5G815Aram contains the UL5 gene with flanking BamHI sites. This plasmid was constructed by ligating a BglII/XhoI fragment from p6UL5G815A (26) containing the carboxyl-terminal portion of the mutant UL5 gene to a BglII/XhoI fragment from the p6UL5Bam vector (31) containing the amino-terminal portion of the wild-type UL5 gene. The mutant UL5G815A gene was then cloned as a 3.3-kb pair BamHI fragment into the pAcCL29 baculovirus transfer vector (32) digested with BamHI. Correct orientation of the mutant UL5 gene with respect to the viral polyhedron promoter was confirmed by restriction enzyme digestion. The pAcUL5G815A DNA was linearized with ScaI and cotransfected with Baculogold® Baculovirus DNA into SF9 cells as described in the manufacturer's instructions. The oligonucleotide used to create the G815A mutation in motif V also created a ScaI site, which was used to confirm the restriction enzyme cleavage site in the UL5 gene (26). Viral stocks were prepared and stored at 4°C as described above.

**Protein Expression and Preparation of Cell Extracts**

Unless stated otherwise, all procedures were performed at 4°C. One liter of SF9 cells was grown in suspension at 27°C in medium supplemented with 0.01% Pluronic® F-68 in a 6-liter flask (90 rpm). When the cell density reached 1 × 106 cells/ml (doubling time no greater than 24 h), the cells were infected with 10 plaque-forming units of the indicated recombinant baculovirus/cell and grown for an additional 55-60 h at 27°C. Cells were harvested at 1000 × g in a GSA rotor, washed with cold serum-free medium, resuspended in 20 ml of ice-cold Buffer A, and allowed to swell on ice for 15 min. Cells were lysed by 15 strokes with a type B pestle. Nuclei were pelleted by centrifugation at 1000 × g. Nuclear extracts were prepared by resuspending the nuclear pellet in Buffer B containing 1.7 M NaCl. Both nuclear and cytosolic extracts were dialyzed against centrifugation at 48,000 × g in an SS34 rotor. Cytosolic extracts containing the UL5-52 subcomplex or the UL8 protein were fractionated by precipitation with 1 M ammonium sulfate on ice for 2 h. Total cellular extracts containing UL29 were fractionated by precipitation with 1 M ammonium sulfate. The ammonium sulfate precipitated protein pellets were resuspended in Buffer B containing 0.1 M NaCl, dialyzed overnight to remove ammonium sulfate, and reclarified by centrifugation at 48,000 × g.

**Additional Protein Purification**

The UL5-52 Subcomplex and UL8—The UL5-52 helicase-prime subcomplex (wild-type or UL5(G815A) variant) and UL8 were purified from ammonium sulfate extracts by chromatography through MonoQ® HR 5/5 anion exchange and Superose® 12 HR. The dialyzed sample was loaded onto the MonoQ® column equilibrated with Buffer B containing 0.1 M NaCl, the column was washed with 2 column volumes of the equilibration buffer, and the protein was eluted using a 20-ml linear gradient of Buffer B containing 0.1 M NaCl. Fractions containing the helicase-prime subcomplex were identified by both ATPase assay and by SDS-polyacrylamide gel electrophoresis. Fractions containing UL8 were identified by SDS-polyacrylamide gel electrophoresis. The clearest fractions containing the protein of interest were pooled, adjusted to 0.2 M NaCl, concentrated to 0.4 ml, and further fractionated by gel filtration through a Superose® 12 HR 10/30 column equilibrated with Buffer B containing 0.2 M NaCl and 0.01% Nonidet P-40. Fractions containing at least 95% homogeneous protein were pooled and frozen in small aliquots at −70°C. Protein concentrations were determined by using the Bio-Rad Protein Assay Reagent.

UL29—UL29 was purified from ammonium sulfate fractions by chromatography through a High Load® 16/10 SP Sepharose® Fast Flow, Superose® 12 HR 10/30, and double-strand DNA cellulose. The dialyzed sample was loaded onto the SP Sepharose® column equilibrated with Buffer B containing 0.1 M NaCl, and the protein was eluted using a 100-ml linear gradient between Buffer B containing 0.1 M NaCl and 0.7 M NaCl. Fractions containing UL29 (determined by SDS-polyacrylamide gel electrophoresis) were pooled, concentrated to 0.4 ml, and further fractionated by gel filtration through a Superose® 12 column equilibrated with Buffer B containing 0.1 M NaCl. Fractions containing UL29 were checked for nuclease activity. UL29 containing fractions were divided into two pools, nuclease-free and nuclease-contaminated. Nuclease-contaminated UL29 fractions were pooled and further fractionated on a 2-ml double-stranded DNA cellulose column (2.55 ml packed bed) in Buffer B containing 0.1 M NaCl. Under these conditions UL29 does not bind to the column, but any remaining nuclease would be expected to bind. Fractions were checked for nuclease, and nuclease-
free fractions were pooled with the nuclease free fractions from the Superose® 12 column and stored in small aliquots at –70 °C.

Enzyme Assays

Direct Primase Assays—RNA primer synthesis reactions (25 μl) contained 40 μM TAPS (pH 8.5), 3.5 mM MgCl₂, 2 mM ATP, 1 mM GTP, 1 mM UTP, 2 mM DTT, 0.1 mg/ml bovine serum albumin, 10% glycerol, 10 μM [α-32P]dCTP (3000 Ci/mmol) (1 pmol (molecules) of a 50-base DNA oligonucleotide template containing a preferred primase initiation site) mapped from pBS plasmid DNA,³ and 2 pmol of the UL5-52 subcomplex (wild-type and UL5(G815A) variant) with or without a 3-fold molar excess of the UL8 protein. Reactions were allowed to proceed for 60 min at 30 °C, stopped by the addition of 1 μl of 0.25 mM EDTA, dried by Speed Vac, and resuspended in 10 μl of 50% formamide and 0.01% bromophen blue. Oligonucleotides were baled for 2 min, and the reaction products were separated on a 18% Long Ranger™/7 M urea gel. The gels were dried and exposed to film.

ATPase Assays—ATPase reactions (50 μl) contained 20 mM Na⁺ HEPES (pH 7.6), 1 mM DTT, 0.1 mg/ml bovine serum albumin, and 10% glycerol. Unless otherwise stated all reactions contained 5 mM NaOH, 1 mM EDTA, 10% glycerol, 0.01% bromocresol green), and either 0.45 pmol of the UL5-UL52 subcomplex or 0.9 pmol of the UL5(G815A)-UL52 subcomplex. Reactions were allowed to proceed for 30 min at 37 °C, and formation of inorganic phosphate was determined by the addition of 0.8 ml of an acidic ammonium molybdate solution containing Malachite green (33). After thoroughly mixing each reaction, color development was quantified by the addition of 0.1 ml of 34 mM sodium citrate, and the absorbance at 660 nm was determined. Nanomoles of inorganic phosphate released per reaction were determined from a standard curve.

Helicase Assays—Helicase reactions (20 μl) contained 20 mM Tris-HCl (pH 7.5), 5 mM DTT, 5 mM MgCl₂, 5 mM ATP, 0.1 mM bovine serum albumin, 10% glycerol, and 55 nmol DNA substrate molecules (diagrammed in Fig. 4A) consisting of a single-stranded M13mp18 molecule singly primed with a 45 base oligodeoxynucleotide primer (22-bases homologous to M13 with a 23-bases nonhomologous 3' tail) (9). Reaction rates containing various amounts of the UL5-UL52 subcomplex were allowed to proceed for 30 min at 34 °C and terminated by the addition of one half volume of stop solution (0.1 M EDTA (pH 8), 40% glycerol, 0.1% bromophen blue). Reaction products were separated by electrophoresis on a 15% nondenaturing acrylamide gel at 150-200 volts at 4 °C until the bromophen blue had migrated approximately half way down the gel. Wet gels were wrapped in plastic and exposed to film overnight.

Leading Strain DNA Replication Assays—Leading strain DNA replication assays were performed as described previously using 25 fmol of pBS+ single-stranded DNA molecules singly primed with a 62-base oligodeoxynucleotide primer (see Fig. 5A, 30 bases homologous to pBS+ with a 32-base nonhomologous 5' tail) (15). The replication fork substrate was generated by incubation of the singly primed DNA with 0.25 pmol HSV-1 polymerase (UL3042) for 15 min. Varying amounts of the UL5-UL52 subcomplex (wild type and variant), a 3-fold molar excess UL8, and 15 pmol of UL29 were then added to the reactions. After 60 min at 30 °C, an equal volume of 1% SDS, 40 mM EDTA, and 1 mg/ml proteinase K was added. After incubation at 37 °C for 60 min, the reaction products were precipitated using 0.04 μg of glycogen, 0.3 M sodium acetate (pH 5.2), and 2 volumes of 100% ethanol at –20 °C. DNA pellets were resuspended in 25 ml of alkaline loading buffer (100 mM NaOH, 1 mM EDTA, 10% glycerol, 0.1% bromocresol green), and reaction products were separated on a 0.8% alkaline agarose gel in 30 mM NaOH, 1 mM EDTA at 25 volts at 4 °C. Gels were neutralized for 30 min in 7% trichloroacetic acid, dried, and exposed to film.

Generation of Figures

Autoradiograms were scanned using the Microtek ScanMaker II on an Apple MacIntosh computer. Photos were generated using Adobe Photoshop™ version 3.0. The sequence alignment of the helicase/primase proteins shown in Fig. 1 was performed using computer software provided by the Genetics Computer Group, Madison, Wisconsin (34, 35).

RESULTS

Generation of Recombinant Baculovirus and Purification of the UL5(G815A)-52 Helicase-Primase Subcomplex—The produ
RNA primase activity of HSV-1 helicase-primase is unaffected by a mutation in motif V of UL5. RNA primase activity on a preferred primase initiation site template catalyzed by wild-type or variant helicase-primase subcomplex was measured as described under “Experimental Procedures” and analyzed by 18% denaturing SDS-polyacrylamide gel electrophoresis and autoradiography. Lane 1 represents a reaction containing only the UL8 protein (6 pmol). Lanes 2 and 3 represent reactions containing 2 pmol of the UL5-52 subcomplex (wild type UL5 or UL5(G815A)) plus a 3-fold molar excess of UL8 (6 pmol). Lanes 4 and 5 represent reactions containing 2 pmol of only the UL5-52 subcomplex only (wild type UL5 or UL5(G815A)).

The values for $K_m$ for ssDNA and ATP of both wild-type and the UL5(G815A)52 subcomplex were compared. ATP hydrolysis followed a linear time course for both enzymes, suggesting that what was being measured were the initial velocities of each reaction. In the presence of saturating levels of ATP, MgCl₂, and ssDNA, the UL5(G815A)52 subcomplex exhibited a 3-4-fold decrease in ssDNA-dependent ATPase activity. Although the DNA-independent ATPase activity of each subcomplex is very low, it appears to be unaffected by the G815A mutation (Fig. 3 and Table 1). These data suggested that the UL5(G815A)52 protein may possess a lower affinity for ssDNA. In order to test this hypothesis, the affinity of each subcomplex was analyzed by the ability to bind to ssDNA cellulose. Both wild-type and UL5(G815A)52 subcomplex bind to a ssDNA cellulose column and elute from the column at a NaCl concentration of 0.2 M (data not shown). Because small changes in the affinity for ssDNA may not be detected by this method, the values for $K_m$ for ssDNA and ATP and turnover ($K_{cat}$) of ATP were also experimentally determined for each helicase-primase subcomplex (Table 1). The G815A residue change in helicase motif V resulted in a 3-4-fold decrease in the value of $K_{cat}$ for ssDNA-dependent ATPase activity but did not seem to alter the value $K_{cat}$ for DNA-independent ATPase activity. The UL5(G815A)52 subcomplex also exhibited a 3-fold increase in the value for $K_m$ for ssM13mp18 DNA and a 1.4-fold increase in the $K_m$ for ATP. The values for $K_m$ (DNA and ATP) and $K_{cat}$ determined for the wild-type subcomplex are consistent with those previously reported for the wild-type heterotrimeric complex (39, 40). These data are consistent with the hypothesis that motif V may play a role in the interaction of the HSV-1 helicase-primase with

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Leading strand DNA synthesis assay requires the helicase to function like the helicase assay, which requires only that the helicase unwind duplex DNA. The 3–4-fold decrease in ssDNA-dependent ATP hydrolysis exhibited by the UL5(G815A)-52 subcomplex suggested that the helicase activity of the enzyme might also be affected by this residue change. To determine if the UL5(G815A)-52 subcomplex retained DNA helicase activity, the abilities of the wild-type and the variant subcomplex to displace a short strand of DNA from a partial duplex substrate were compared. In this assay, various amounts of helicase-primase subcomplex were incubated with the radiolabeled partial duplex DNA substrate (Fig. 4A) in the presence of ATP and MgCl₂, and the reaction products were separated by native gel electrophoresis. Comparison of the intensity of the bands corresponding to annealed oligo (Fig. 4B, top band) versus the displaced oligo (Fig. 4B, bottom band) indicated that the helicase activity of the UL5(G815A)-52 subcomplex is equivalent to the wild-type subcomplex. Thus, the decrease in the ability to hydrolyze ATP in the presence of ssDNA exhibited by the UL5(G815A)-52 subcomplex (Fig. 3, and Table I) is not reflected in the ability of the enzyme to unwind a short region of double-stranded DNA (Fig. 4B).

Glycine 815 in Motif V Is Not Required for Non-origin-dependent Leading Strand DNA Synthesis in vitro—All of the assays described previously measure properties of the helicase-primase subcomplex that are independent of the other HSV-1 DNA replication proteins. The ability to replicate a DNA substrate containing a preformed replication fork in vitro (Fig. 5A) requires each of the seven essential HSV-1 replication proteins: DNA polymerase (UL30-42), ssDNA-binding protein (UL29), and the heterotrimeric helicase-primase (UL5-52) (15). Unlike the helicase assay, which requires only that the helicase unwind a very short stretch of duplex DNA (22 bases), the leading strand DNA synthesis assay requires the helicase to unwind long stretches of duplex DNA (up to 3 kb) and may also depend on protein-protein interactions with the other members of the HSV-1 replication machinery. Whereas the UL5-52 subcomplex can catalyze both ATP hydrolysis and duplex DNA unwinding, in vitro leading strand DNA synthesis also requires the UL8 subunit of the helicase-primase complex (15).

To determine if the UL5(G815A) protein can participate in leading strand DNA synthesis, both the wild-type and the UL5(G815A)-52 subcomplex were incubated with the preformed fork substrate (Fig. 5A), UL8, HSV-1 polymerase (UL30-42), and ssDNA-binding protein (UL29) for various amounts of time. The subsequent reaction products were analyzed on an alkaline agarose gel and visualized by autoradiography. As shown in Fig. 5B (lane 1), in the absence of the UL5-52 subcomplex, the predominant products of the reaction were linear DNA molecules approximately 3 kb in size that represent extension of the primer once around the circular single-stranded template by a combination of the DNA polymerase and UL29. In the absence of the UL8 protein, the products of the reaction consisted of a mixture of 3-kb and approximately 6-kb linear molecules. The origin of these 6-kb products was not investigated in detail, but it is likely that they result from extension of short hairpins formed at the 3' end of template DNA containing a single random nick caused by a small amount of nuclease contamination in the preparations of UL5-52 subcomplex (41). In the presence of UL8, both the wild-type and the variant subcomplex support the synthesis of reaction products at least 12 kb in length (Fig. 5B, lanes 4–6 and lanes 7–9), which presumably arise via rolling circle type strand displacement synthesis. It has previously been shown...
that this displacement synthesis is dependent on the helicase activity of the HSV-1 helicase-primase complex (15). In summary, although the UL5(G815A) protein was shown to be incapable of supporting origin-dependent DNA replication in vivo (26), replacement of Gly$^{115}$ in motif V of UL5 with an Ala residue does not have a substantial effect on strand displacement DNA synthesis in vitro.

**DISCUSSION**

Genetic studies have indicated that the most conserved residues in the six helicase motifs in UL5 are required to support HSV-1 DNA replication in vivo (26). We have characterized the biochemical properties of HSV-1 helicase-primase containing a mutation in a conserved glycine in motif V of UL5. Although one possible effect of this mutation is a change in conformation, it has previously been demonstrated that UL5(G815A) can be expressed as a stable protein in a transient transfection assay (26). Furthermore, the studies reported herein reveal that purified UL5(G815A)-52 subcomplex is as stable as wild-type and retains primase, ssDNA binding, and strand displacement activities in vitro. The variant subcomplex also retains the ability to support non-origin-dependent leading strand DNA synthesis in the presence of the other HSV-1 replication proteins. Thus, it is unlikely that the overall conformation of the UL5 protein is significantly altered by this amino acid substitution.

Several possibilities have been considered to explain the in vivo defect of the motif V UL5 variant helicase. Although the DNA-independent ATPase activity of the variant and wild-type enzyme were indistinguishable, the UL5(G815A)-52 subcomplex displayed both a higher $K_m$ for ssDNA and lower maximal rate of ssDNA-dependent ATP hydrolysis (Table I). In order to unwind duplex DNA, a helicase must interact with the DNA substrate, suggesting that one or more domains responsible for contacting DNA might be conserved among these proteins. It is possible that the in vivo defect of the motif V variant may be due to a decreased affinity of the helicase for ssDNA. A decreased affinity for DNA could inherently affect the ability of the helicase to translocate processively along DNA and melt the DNA duplex. Most DNA helicases involved in DNA replication such as SV40 TAg, T7 gp4, T4 gp41, and E. coli DnaB are highly processive (reviewed in Refs. 1 and 42). Processive unwinding activity may require a helicase to bind tightly to its DNA substrate. Because the in vitro leading strand DNA replication assay shown in Fig. 5B uses an excess of the helicase-primase complex to DNA, the UL5 helicase may not be required to act processively under these conditions. Analysis of the processivity of the wild-type and motif V variant helicases will require further investigation. However, it is possible that motif V plays a role in binding to ssDNA and/or processivity of helicase activity that is not directly measured in our in vitro assays.

Duplex DNA unwinding by a helicase requires energy, presumably provided by ATP hydrolysis. The UL5(G815A)-52 subcomplex is compromised in the induction of ssDNA-dependent ATP hydrolysis but not in the ability to unwind DNA in either of our in vitro assays (Figs. 4 and 5). It is possible that the in vitro assays currently used to test helicase function do not accurately reflect the in vivo requirements for the function of the UL5 protein. For instance, although the variant helicase-primase subcomplex can catalyze strand displacement DNA synthesis in vitro, it appears that this assay only measures leading strand DNA synthesis (15). Synthesis of DNA in vivo may require a complex assembly of proteins to allow synthesis on both the leading and lagging DNA strands. The UL5 helicase may play a role in the coordination of leading and lagging strand synthesis. This has been shown to be true for the T7 gp4 helicase-primase (43). It is possible that motif V plays a role in allowing the HSV-1 helicase-primase to catalyze efficient coordination of leading and lagging strand DNA synthesis. Because HSV-1 lagging strand DNA synthesis has not been reconstituted in vitro, this hypothesis remains to be tested.

HSV-1 DNA replication is believed to initiate at a viral origin of DNA synthesis. Because the in vitro strand displacement DNA synthesis assay used in our experiments is not dependent on a viral origin of replication, another possible role for UL5 is to aid in catalyzing origin-dependent DNA synthesis. Although an in vitro origin-dependent HSV-1 DNA replication assay has not been reconstituted to date, this hypothesis is consistent with the inability of the motif V variant to support replication of a origin containing plasmid in vivo (26). We cannot rule out the possibility that motif V may be required for UL5 to participate in origin-dependent DNA replication due to an interaction with one or more members of the HSV-1 replication machinery at the viral origin of replication. How-
ever, because motif V is also present in many helicases not required for DNA replication, a role for this conserved motif in a specific interaction with other replication proteins at the origin of replication is somewhat unlikely.

Although the exact mechanism of HSV-1 DNA replication is not understood, current models suggest that like T4, the processes of HSV-1 DNA replication and recombination may be closely linked (reviewed in Ref. 44). The conservation of sequences between UL5 and other helicases involved in DNA repair and recombination suggests that the UL5 helicase may also play a role in recombination. The E. coli helicases UvrAB and Rep are involved in DNA repair, whereas the RecBC complex is involved in homologous recombination (reviewed in Refs. 1–3). The PIF helicase is involved in yeast mitochondrial DNA repair, whereas the RecBC complex is involved in recombination. The RecBC complex is also implicated in the repair of DNA damage (reviewed in Refs. 1–3). The conservation of sequences between UL5 and other helicases involved in DNA replication and recombination may be important for a function of the UL5 protein in recombination and/or DNA repair.

In summary, we have demonstrated that the conserved glycine residue in motif V (Gly\textsuperscript{815}) is required for maximal induction of ssDNA-dependent ATP hydrolysis catalyzed by the UL5-52 subcomplex in vitro. It is possible that the in vivo effect exhibited by this variant helicase protein is a consequence of a reduced affinity for DNA resulting in a less processive helicase. Alternatively, we cannot rule out the possibility that the motif V variant helicase is defective in other aspects of HSV-1 DNA replication (such as coordination of leading and lagging strand DNA synthesis and/or protein-protein interactions) that may not be reflected in the in vitro assays used in these experiments.

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