ICP8 Enhances the Ability of the Viral DNA Helicase-primase to Unwind Cisplatin-modified DNA

The herpes simplex virus type-1 UL5, UL8, and UL52 genes encode an essential heterotrimeric DNA helicase-primase that is responsible for concomitant DNA unwinding and primer synthesis at the viral DNA replication fork. The viral single-strand DNA-binding protein (ICP8) can stimulate DNA unwinding by the helicase-primase as a result of a physical interaction that is mediated by the UL8 subunit. In this study, we investigated the ability of the helicase-primase to unwind a fork-like substrate that contains an intrastrand d(GpG) DNA cross-link produced by the antitumor drug cisplatin. We also examined the ability of ICP8 to modify the effect of the cisplatin lesion. The data show that the lesion inhibited the helicase-primase when located on the DNA strand along which it translocates. However, the lesion did not represent a permanent obstacle to its progression. In contrast, the adduct did not affect the helicase-primase when located on the opposite DNA strand. ICP8 specifically stimulated DNA unwinding by the helicase-primase. Coating concentrations of ICP8 were necessary for optimal unwinding of damaged DNA. Addition of competitor DNA to helicase reactions led to substantial reduction of DNA unwinding by the helicase-primase, suggesting that the enzyme is distributive. ICP8 did not abolish the competition, indicating that it did not stimulate the helicase by increasing its processivity. Rather, ICP8 may stimulate DNA unwinding and enable bypass of cisplatin damaged DNA by recruiting the helicase-primase to the DNA.

The most abundant adduct generated by the interaction of the antitumor drug cis-diaminedichloroplatinum (II) (cis-DDP) with DNA is the 1–2-intrastrand d(GpG) cross-link (cis-DDP-d(GpG))(1). The capacity of this adduct to affect cellular DNA replication significantly contributes to the cytotoxicity and mutagenicity of cisplatin (2, 3). The effects of intrastrand cisplatin lesions on several DNA helicases implicated in DNA recombination and repair have been examined (4–6). In addition, we have recently studied the effect of a site-specific cis-DDP-d(GpG) adduct on a DNA helicase that is required for DNA replication, the herpes simplex virus type-1 (HSV-1) origin-binding protein (UL9 protein)(7). In this study, we have examined the effect of a site-specific cis-DDP-d(GpG) adduct on the helicase activity of the HSV-1 DNA helicase-primase. Unlike the UL9 protein, which is required for origin-specific DNA unwinding during replication initiation (8–10), the helicase-primase translocates along the lagging strand, unwinding the DNA at the replication fork (8, 11). The helicase-primase 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 (8). A third subunit, the 80-kDa UL8 gene product, is an essential component of the helicase-primase complex. Its precise role is still unclear, but it has been reported to stimulate both the primase and helicase activities of the core enzyme in the presence of the HSV-1 single-strand DNA-binding protein, ICP8 (12, 13). These studies predicted an interaction between ICP8 and the helicase-primase heterotrimer mediated by the UL8 protein, which has subsequently been demonstrated (14).

To study the effect of a cisplatin lesion on the progression of a DNA helicase at the replication fork, we examined the activity of the HSV-1 helicase-primase holoenzyme with a fork-like DNA substrate containing a site-specific cis-DDP-d(GpG) adduct. We also examined the ability of ICP8 to modulate the effect of the cisplatin lesion. The results show that the activity of the helicase-primase was inhibited by the adduct. ICP8 significantly increased the activity of the helicase-primase with cisplatin-damaged DNA. We propose that ICP8 stimulates translesion DNA unwinding by recruiting the helicase-primase to the damaged DNA.

MATERIALS AND METHODS

Proteins—ICP8, UL5/52 core enzyme, and UL8 protein were purified to near homogeneity as described (12). Escherichia coli SSB was purchased from Amersham Pharmacia Biotech. T4 polynucleotide kinase was obtained from New England Biolabs, Inc. Restriction endonucleases HaeIII and EcoI and proteinase K were purchased from Boehringer Mannheim.

Chemicals—cis-diaminedichloroplatinum (II), 99.99% pure, was purchased from Aldrich. ATP was from Boehringer Mannheim. 

DNA Substrates—Unmodified or cisplatin-modified substrates B and C (Fig. 1) were constructed as described (15). The oligodeoxyribonucleotides employed to construct substrate A were synthesized on an Applied Biosystem DNA synthesizer and purified by electrophoresis through denaturing polyacrylamide gels. To obtain unmodified substrate A, equimolar concentrations of 90- and 59-mers were annealed to produce the Y-shaped partially duplex molecule. The 59-mer necessary for the construction of platinated substrate A was obtained by ligation of a 22-mer (residues 1–22, starting from the 3′-end of the 59-mer), a

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platinated 12-mer (residues 23–34), and a 25-mer (residues 35–59). Cisplatin modification of the 12-mer (3'-TCCGGTCCCTTT-5'), which contains only one d(GpG) modification site for cisplatin, was performed as follows: cisplatin was dissolved in 5 mM sodium perchlorate at a concentration of 0.5 mg/ml and then incubated in 5 mM Tris-HCl, pH 7.8, 1 mM sodium perchlorate at a 2-fold molar excess with the 12-mer for 48 h at 37 °C. The platinated 12-mer was then precipitated twice in ethanol in order to remove the unreacted drug, and its concentration determined by absorbance at 260 nm. The platinated 12-mer was ligated to the 22- and 25-mers using a 41-mer (complementary to residues 9–49 of the 59-mer) as a scaffold in a reaction containing 10 units of T4 DNA ligase per 5'-end. The resulting platinated 59-mer was purified by denaturing polyacrylamide gel electrophoresis, 32P-labeled, and hybridized to an equimolar concentration of the complementary 90-mer to yield platinated substrate A.

Fig. 1. DNA substrates used in this study. Underlined GG residues indicate the target site for cis-DDP modification. Boxes show the HaeIII site in A (substrate A) and the AciI site in B and C (substrates B and C, respectively).

To determine the extent of cisplatin modification of substrate A, we took advantage of the fact that the modification site is part of a HaeIII restriction endonuclease site, which upon modification is rendered resistant to cleavage by the enzyme (4). 5 ng of 32P-labeled unmodified or platinated substrate A was digested with 10 units of HaeIII under standard conditions. Fig. 2 shows that the vast majority of the platinated 90/59-mer was resistant to cleavage, whereas the unmodified substrate was digested nearly to completion. PhosphorImager quantitation indicated that 95% of the platinated substrate was resistant to HaeIII cleavage and therefore contained the cis-DDP-d(GpG) adduct. Similar experiments performed with substrates B and C, and AciI restriction endonuclease indicated that 90% of the substrates were platinated (data not shown).

Helicase Assay—DNA helicase reactions were performed at 37 °C in 20 mM HEPES-NaOH, pH 7.5, 1 mM dithiothreitol, 10% glycerol, 4.5 mM MgCl2, 3 mM ATP, 0.1 mg/ml bovine serum albumin. Incubation times, DNA helicase-primase (equimolar concentrations of UL5/52 and UL8 proteins), ICP8, E. coli SSB, and DNA substrate concentrations were as indicated in the figure legends. Assays included a 10–40-fold molar excess of unlabeled 28-mer or 27-mer to prevent reannealing of the unwind DNA strand. In control helicase assays, performed in the absence of 28-mer, DNA unwinding was only 30% of the reaction with the 28-mer (data not shown). The reactions were terminated by addition
of 0.2 volumes of 150 mM EDTA, 1% SDS and then digested for 20 min at 37 °C with 2 mg/ml proteinase K. 3 μl of 0.5% bromphenol blue and 40% glycerol were then added, and the reaction mixtures were resolved by electrophoresis through 15% nondenaturing polyacrylamide gels. The gels were scanned using a PhosphorImager, and the release of single-stranded DNA was quantitated using the ImageQuant software. DNA unwinding activity is expressed as the percentage of displaced single-stranded DNA was quantitated using the ImageQuant software. The gels were scanned using a PhosphorImager, and the release of single-stranded DNA was quantitated using the ImageQuant software.

**Results**

**Effect of the cis-DDP-d(GpG) Adduct on DNA Unwinding by the Helicase-primase**—The HSV-1 DNA helicase-primase is believed to be responsible for unwinding DNA at the replication fork (8, 11). The enzyme requires a single-stranded DNA loading site in order to initiate DNA unwinding in the 5′ to 3′ direction (11). We define the strand to which the helicase binds and along which it translocates as the template strand. As shown in Figs. 3–6, and in agreement with our previously published work (12), the helicase-primase can unwind the fork-like substrates depicted in Fig. 1. In order to obtain efficient unwinding and prevent reannealing of the unwound, labeled primer strand, an excess of unlabeled oligodeoxyribonucleotide, complementary to the double-stranded regions of substrates A, B, or C, was present. Consistent with previous reports (7, 12, 16), we found that a 10–40-fold molar excess of unlabeled oligodeoxyribonucleotide was necessary to achieve optimal unwinding of the substrates.

The presence of a single cis-DDP-d(GpG) adduct on the 59-mer template strand inhibited DNA unwinding by the helicase-primase (Fig. 3). As shown in Fig. 3, increasing DNA unwinding activity was observed with both unmodified and platinated DNA, reaching a plateau at ~250 nM enzyme. However, the presence of the cis-DDP-d(GpG) adduct reduced DNA unwinding by a factor of 3–4, and the extent of inhibition remained unchanged over a range of enzyme concentrations from 50 to 500 nM. It is possible that a significant fraction of the total DNA unwinding observed with the platinated substrate may be due to the presence of a small percentage (~5%) of unmodified substrate (see under “Materials and Methods”). The appearance of a faint band at an intermediate position between substrate and product was occasionally observed with the platinated DNA (data not shown). We believe that this band represents a hairpin structure that is induced by the cis-DDP-d(GpG) adduct in a fraction of the 59-mer population, thus preventing its hybridization to the 90-mer oligodeoxyribonucleotide. The capacity of cisplatin to induce such hairpin structures in oligodeoxyribonucleotides has been documented (17). Fig. 4 shows that the lesion inhibited DNA unwinding during the entire course of the reaction, suggesting that the adduct blocks helicase action. However, the lesion did not represent a permanent obstacle to the helicase-primase because addition of fresh enzyme resulted in increased DNA unwinding activity (Fig. 5).

The data in Fig. 5 also show that the helicase-primase is unstable during the course of the reaction because addition of fresh enzyme resulted in increased DNA unwinding activity. Preincubation of the helicase-primase in the absence of DNA substrate showed that its half-life was approximately 30 min at 37 °C. In addition, we observed that preincubation of the helicase-primase with DNA substrate significantly increased its stability. In contrast, ICP8, in either the absence or presence of DNA substrate, was not able to increase the stability of the helicase-primase (data not shown).

In contrast to the data obtained with substrate A, the helicase-primase unwound unmodified and cisplatin-modified substrates B and C with comparable efficiency (Fig. 6). As can be seen, regardless of whether the lesion was placed 13 or 7 nucleotides from the fork junction (substrates B and C, respectively), its position on the primer strand had no effect on the activity of the helicase-primase. We found that the helicase-

**FIG. 2.** Extent of cis-DDP-modification of substrate A. Unmodified or cisplatin-modified substrate A was digested with HaeIII as described under “Materials and Methods.” The arrow indicates the position of the HaeIII fragment. Pt, cisplatin-modified DNA.

**FIG. 3.** Effect of the cis-DDP-d(GpG) adduct on the helicase activity of the helicase-primase: DNA unwinding of substrate A as a function of protein concentration. Unmodified or cis-DDP-modified substrate A (11 nM molecules) was incubated for 60 min as described under “Materials and Methods” with the indicated concentrations of helicase-primase and a 10-fold molar excess of unlabeled 28-mer, A, autoradiogram of the reaction products; lanes 1–8, unmodified substrate A. Lane 1, no protein; lanes 2–7, 12.5, 25, 50, 100, 250, and 500 nM helicase-primase, respectively; lane 8, heat-denatured substrate. Lanes 9–16, cis-DDP-modified substrate A. Lane 9, no protein; lanes 10–16, 12.5, 25, 50, 100, 250, and 500 nM helicase-primase, respectively; lane 16, heat-denatured substrate. The positions of the 90/59-mer substrates and of the unwound 59-mers are as indicated. B, quantitation of the data shown in A. ○, unmodified DNA; ▲, cis-DDP-modified DNA.
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Fig. 4. Effect of the cis-DDP-d(GpG) adduct on the helicase activity of the helicase-primase: kinetics of DNA unwinding. Unmodified or cis-DDP-modified DNA substrate A (11 nM molecules) was incubated with 250 nM helicase-primase and a 10-fold molar excess of unlabeled 28-mer as described under “Materials and Methods.” At the indicated times, reactions were stopped and helicase activity measured. ○, unmodified DNA; ▲, cis-DDP-modified DNA.

Fig. 5. Effect of the addition of fresh helicase-primase on the unwinding of unmodified and cis-DDP-modified substrate A. Unmodified or cis-DDP-modified substrate A (8.5 nM molecules) was incubated with 250 nM helicase-primase and a 20-fold molar excess of unlabeled 28-mer as described under “Materials and Methods.” At the time indicated by the arrows (30 min), additional 250 nM helicase-primase was added to both unmodified and cis-DDP-modified substrates. At the indicated times, reactions were stopped, and helicase activity was measured. ○, unmodified DNA; □, unmodified DNA after addition of fresh enzyme; ▲, cis-DDP-modified DNA; ◆, cis-DDP-modified DNA after addition of fresh enzyme.

The data in the preceding section indicate that the major cisplatin DNA lesion, when present on the template strand, inhibits the helicase-primase only when it is present on the substrate and that it does not prevent dissociation of the helicase-primase. Because ICP8 has been shown to possess heliXdestabilizing activity (18), we determined its capacity to unwind substrate A under our experimental conditions. As can be seen in Fig. 7, a low level of displacement was detected only at 1000 and 1200 nM ICP8 for the unmodified and cisplatin-modified substrates, respectively. Quantitation by Phosphoimager analysis indicated that the amount of displacement was 10 and 15% for 1000 and 1200 nM ICP8, respectively. Estimates of the DNA-binding site size of ICP8 fall in the range of 1 ICP8 to 12–22 nucleotides of single-stranded DNA (8, 18–20). By assuming an ICP8 binding site size of 14–15 nucleotides and by considering the total concentration of DNA (substrate plus unlabeled 28-mer) in the reaction, we estimated the concentration of ICP8 necessary to completely cover the DNA substrate (coating concentration) was ~800 nM. As shown in Fig. 7 (lanes 4 and 11), no strand-displacement activity was detected at this concentration of ICP8.

Fig. 8 shows that addition of ICP8 stimulated the DNA unwinding activity of the helicase-primase with platinated DNA up to 6-fold. Maximal stimulation was observed at coating concentrations of ICP8 (800 nM). The stimulatory effect of ICP8 on the helicase-primase is likely to be a consequence of specific protein-protein interactions because it was not observed in the presence of the heterologous E. coli SSB (see Fig. 8, columns 11–20).

Effect of competitor DNA on the unwinding of unmodified or platinated substrate A by the helicase-primase in the presence or absence of ICP8—Addition of a 4-fold molar excess of unlabeled substrate A to an ongoing helicase reaction with unmodified substrate A in which the concentration of single-stranded 5’ DNA ends was in excess over helicase-primase resulted in a significant decrease in DNA unwinding activity (Fig. 9A). This result suggests that the helicase-primase readily dissociates from the DNA substrate and that it is distributive. A higher concentration of unlabeled 28-mer (40-fold molar excess) was used in the competition experiments in order to prevent reannealing of the unwound labeled DNA strand in the presence of the competitor DNA.

To investigate the mechanism by which ICP8 stimulates DNA unwinding, we performed competition experiments in the presence of coating concentrations of ICP8. Fig. 9B shows that addition of ICP8 to a helicase reaction with unmodified substrate A did not significantly alter the level of competition, suggesting that it does not prevent dissociation of the helicase-primase from the substrate and that it does not stimulate the helicase-primase by increasing its processivity.

We then examined the effect of coating concentrations of ICP8 in competition experiments performed with cisplatin-modified substrate A. We observed an increased level of competition with the platinated DNA compared with the unmodified substrate (compare Fig. 9, B and C), suggesting that the lesion induces the helicase-primase to dissociate rapidly, even in the presence of ICP8.2 Consistent with this conclusion, Fig. 10 shows that even in the presence of ICP8, the helicase-primase was more efficiently competed from the platinated DNA than from the unmodified DNA substrate, thus confirming that the cis-DDP-d(GpG) adduct results in dissociation of

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1 Competition experiments with platinated substrate A in the absence of ICP8 could not be performed due to the inhibition imposed on the helicase-primase by the lesion.

2 Competition experiments with platinated substrate A in the absence of ICP8 could not be performed due to the inhibition imposed on the helicase-primase by the lesion.
the helicase-primase. In addition, the data in Fig. 10 reiterate that increasing concentrations of competitor DNA led to a similar reduction in DNA unwinding activity with unmodified substrate A both in the absence or presence of ICP8.

**DISCUSSION**

In this work, we have studied the capacity of a replicative DNA helicase, the HSV-1 DNA helicase-primase, to unwind fork-like DNA substrates that contain the major cisplatin-DNA adduct, cis-DDP-d(GpG). The role of the HSV-1 single-strand DNA-binding protein, ICP8, in modulating the activity of the HSV-1 SSB Facilitates Translesion Bypass by Helicase-primase 13805

**FIG. 6.** Effect of the cis-DDP-d(GpG) adduct on the helicase activity of the helicase-primase: strand specificity. A, unmodified or cis-DDP-modified DNA substrate B (11 nM molecules) was incubated for 60 min as described under “Materials and Methods” with the indicated concentrations of helicase-primase and a 20-fold molar excess of unlabeled 27-mer. B, unmodified or cis-DDP-modified DNA substrate B (11 nM molecules) was incubated with 250 nM helicase-primase and a 20-fold molar excess of unlabeled 27-mer as described under “Materials and Methods.” At the indicated times, reactions were stopped, and helicase activity was measured. C, unmodified or cis-DDP-modified DNA substrate C (11 nM molecules) was incubated for 30 min as described under “Materials and Methods” with the indicated concentrations of helicase-primase and a 20-fold molar excess of unlabeled 27-mer. D, unmodified or cis-DDP-modified DNA substrate C (11 nM molecules) was incubated with 400 nM helicase-primase and a 20-fold molar excess of unlabeled 27-mer as described under “Materials and Methods.” At the indicated times, reactions were stopped, and helicase activity was measured. E, unmodified DNA; ▲, cis-DDP modified DNA.

**FIG. 7.** Unwinding of unmodified or cis-DDP-modified substrate A by ICP8. Unmodified or cis-DDP-modified substrate A (8.5 nM molecules) was incubated for 30 min as described under “Materials and Methods” with a 40-fold molar excess of unlabeled 28-mer and the indicated concentrations of ICP8. Lanes 1–7, unmodified substrate A. Lane 1, no protein; lane 2, 200 nM ICP8; lane 3, 600 nM ICP8; lane 4, 800 nM ICP8; lane 5, 1000 nM ICP8; lane 6, 1200 nM ICP8; lane 7, heat-denatured substrate. Lanes 8–14, cis-DDP-modified substrate A. Lane 8, no protein; lane 9, 400 nM ICP8; lane 10, 600 nM ICP8; lane 11, 800 nM ICP8; lane 12, 1000 nM ICP8; lane 13, 1200 nM ICP8; lane 14, heat-denatured substrate.

**FIG. 8.** Effect of ICP8 and *E. coli* SSB on DNA unwinding by the helicase-primase. Unmodified (■) or cis-DDP-modified (□) DNA substrate A (8.5 nM molecules) was incubated for 30 min as described under “Materials and Methods” with 250 nM helicase-primase, a 40-fold molar excess of unlabeled 28-mer, and the following concentrations of ICP8 or *E. coli* SSB. Columns 1, 6, 11, and 16, helicase-primase alone; columns 2 and 7, helicase-primase plus 200 nM ICP8; columns 3 and 8, helicase-primase plus 400 nM ICP8; columns 4 and 9, helicase-primase plus 600 nM ICP8; columns 5 and 10, helicase-primase plus 800 nM ICP8; columns 12 and 17, helicase-primase plus 200 nM *E. coli* SSB; columns 13 and 18, helicase-primase plus 400 nM *E. coli* SSB; columns 14 and 19, helicase-primase plus 600 nM *E. coli* SSB; columns 15 and 20, helicase-primase plus 800 nM *E. coli* SSB.
helicase-primase was also examined. Our results show that the DNA unwinding activity of the helicase-primase was significantly reduced when the \( \text{cis-DPP-d(GpG)} \) adduct was located on the template strand to which the enzyme binds. In contrast, a lesion located on the opposite strand did not affect DNA unwinding (Figs. 3–6). Inhibition was observed throughout the time course of DNA unwinding and at high (500 nM) enzyme concentrations. A comparison of these results with previously published data (7) shows that the activity of the helicase-primase was inhibited more strongly by the \( \text{cis-DPP-d(GpG)} \) adduct than the helicase activity of the HSV-1 origin-binding protein (UL9 protein), the action of which only appeared to be retarded by the lesion. This difference in behavior may reflect the capacity of UL9 protein to oligomerize into a large complex that is capable of unwinding past the lesion. However, even in the case of the helicase-primase, the \( \text{cis-DPP-d(GpG)} \) adduct did not represent an impassable obstacle, because addition of fresh enzyme during the course of the reaction resulted in increased DNA unwinding (Fig. 5).

Our results show that the DNA unwinding activity of the helicase-primase was significantly reduced when the \( \text{cis-DPP-d(GpG)} \) adduct was located on the template strand to which the enzyme binds. In contrast, a lesion located on the opposite strand did not affect DNA unwinding (Figs. 3–6). Inhibition was observed throughout the time course of DNA unwinding and at high (500 nM) enzyme concentrations. A comparison of these results with previously published data (7) shows that the activity of the helicase-primase was inhibited more strongly by the \( \text{cis-DPP-d(GpG)} \) adduct than the helicase activity of the HSV-1 origin-binding protein (UL9 protein), the action of which only appeared to be retarded by the lesion. This difference in behavior may reflect the capacity of UL9 protein to oligomerize into a large complex that is capable of unwinding past the lesion. However, even in the case of the helicase-primase, the \( \text{cis-DPP-d(GpG)} \) adduct did not represent an impassable obstacle, because addition of fresh enzyme during the course of the reaction resulted in increased DNA unwinding (Fig. 5). It is interesting to note that, consistent with our results, it has recently been shown that the DNA unwinding activity of the helicase-primase is also inhibited by two UV-induced DNA lesions, the \( \text{cis-syn cyclobutane thymine dimer} \) and the \( \text{(6–4) thymine-thymine lesion} \), when located on the template strand of the DNA substrate but not on the primer strand.3

3 X. Veaute, G. Giglia, C. W. Lawrence, and A. Sarasin, submitted for publication.
Previous studies have shown the existence of functional and physical interactions between ICP8 and the helicase-primase (12–14). Therefore, we examined the effects of ICP8 on the unwinding of unmodified and platinated substrates by the helicase-primase. Addition of equimolar ICP8 stimulated the helicase-primase on unmodified DNA and significantly reduced the inhibitory effect of the cis-DDP-d(GpG) adduct on platednated DNA (Fig. 8). However, maximal stimulation of the helicase-primase on damaged DNA was observed only with coating concentrations of ICP8 (Fig. 8). The requirement for coating concentrations of ICP8 may be explained by the fact that the lesion perturbs the structure of the DNA (21–23) and presumably obstructs the recognition of the substrate by the helicase-primase. Accordingly, high concentrations of ICP8 would be necessary to recruit the helicase-primase to the site of the cis-DDP-d(GpG) adduct and to allow efficient unwinding. The stimulation was specific for ICP8 because no such effect was observed with a heterologous SSB, E. coli SSB, suggesting that it involves specific protein-protein interactions.

To obtain insight into the mechanism by which ICP8 stimulates the helicase-primase, we performed the competition experiments depicted in Figs. 9 and 10. Addition of excess challenger DNA to ongoing helicase reactions with unmodified substrates, we performed the competition experiment depicted in Fig. 9A. It has recently been suggested that ICP8 stimulates the DNA helicase activity of the UL9 protein by preventing its dissociation from the DNA substrate, thereby increasing its processivity (24). A similar mechanism may explain the stimulatory effect of ICP8 on the helicase-primase. However, we found that even high concentrations of ICP8 failed to prevent the dissociation of the helicase-primase from its substrate (Fig. 9B). Moreover, the level of competition was similar in the absence or presence of ICP8 (Fig. 10), indicating that ICP8 does not increase the processivity of the helicase-primase.

Interestingly, the level of competition observed with the platinated substrate in the presence of ICP8 was greater than for the unmodified substrate (Figs. 9C and 10). These results suggest that the cis-DDP-d(GpG) lesion increases the dissociation rate of the helicase-primase from the DNA. Taken together with the relatively short half-life of the helicase-primase in the absence of DNA, these findings may in part explain the inhibitory nature of the cis-DDP-d(GpG) lesion (Figs. 3–5).

It is possible that ICP8 stimulates DNA unwinding by increasing the stability of the helicase-primase. However, we found no evidence in support of this mechanism. Rather, we favor a model in which ICP8 recruits the helicase-primase by direct protein-protein interactions (14), thereby increasing the association rate of the helicase-primase with DNA and allowing it to unwind the platinated or unmodified DNA. The salient features of our model are depicted in Fig. 11. In the absence of ICP8, the distributive nature of the helicase-primase causes the enzyme to cycle on and off unmodified DNA substrate. The presence of a cis-DDP-d(GpG) lesion induces the enzyme to dissociate and also prevents the helicase-primase from binding to the substrate. In the presence of ICP8, specific protein-protein interactions between ICP8 and the helicase-primase (14) lead to recruitment of the helicase-primase to the ICP8-covered DNA substrate. Recruitment of the helicase-primase to the DNA substrate also occurs with the cisplatin-damaged DNA, thereby permitting the enzyme to unwind past the lesion.

Because DNA helicases are among the first components of the DNA replication fork machinery that would encounter a site of DNA damage, it is important to understand how these enzymes interact with specific DNA lesions. Our previous (7) and current studies show that specific interactions between the helicase-primase or UL9 protein DNA helicase and ICP8 allow unwinding of cisplatin damaged DNA. In HSV-1 DNA replication, it has been shown that specific protein-protein interactions between ICP8, UL9 protein, and the subunits of the helicase-primase are required for the assembly of these proteins into prereplicative sites and that recruitment of the HSV-1 DNA polymerase into these sites is mediated by the UL42 subunit of the DNA polymerase (25, 26). In addition, the recent finding of an interaction between the UL8 subunit of the helicase-primase and the UL30 subunit of the DNA polymerase (27) and the known interaction of ICP8 with both enzymes (14, 28–31) suggest that a complex of DNA polymerase, helicase-primase, and ICP8 may function at the replication fork and may eventually lead to some replicative bypass of a cis-DDP-d(GpG) adduct.

REFERENCES
Herpes Simplex Virus Type-1 Single-strand DNA-binding Protein (ICP8) Enhances the Ability of the Viral DNA Helicase-primase to Unwind Cisplatin-modified DNA
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