Involvement of DNA Polymerase α in Host Cell Reactivation of UV-Irradiated Herpes Simplex Virus

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Aphidicolin is a potent inhibitor of both host cell DNA polymerase α and herpes simplex virus (HSV)-induced DNA polymerase but has no effect on DNA polymerases β and γ of host cells. By using an aphidicolin-resistant mutant (Aph') of HSV, a possible involvement of DNA polymerase α in host cell reactivation of UV-damaged HSV was studied. Plaque formation by UV-irradiated Aph' was markedly inhibited by 1 μg of aphidicolin per ml, which did not affect the plating efficiency of nonirradiated Aph'. Aphidicolin added before 12 h postinfection inhibited plaque formation by irradiated Aph', which became aphidicolin insensitive after 36 h postinfection. The results strongly suggest that host cell DNA polymerase α is involved in the repair of UV-irradiated HSV DNA.

Mammalian cells possess the ability to reactivate UV-irradiated DNA viruses such as simian virus 40 (1, 22), adenoviruses (4, 5), and herpesviruses (7, 13, 18). This phenomenon, referred to as host cell reactivation (HCR), has been used in DNA repair studies because HCR is highly dependent on the ability of the host cells to repair damaged DNA (4, 7). However, the enzymatic mechanisms of HCR are not well understood; the DNA polymerase responsible for HCR has not been identified. Cellular DNA polymerase β has been implicated in DNA repair synthesis (2, 10, 26), and recent studies have led to conflicting conclusions on the role of DNA polymerase α in repair synthesis (3, 6, 15, 23, 34). This study was undertaken to determine whether host cell DNA polymerase α is involved in HCR of UV-irradiated herpes simplex virus (HSV).

Human embryonic fibroblasts (HEF) were prepared as described previously (17) and grown in Eagle minimal essential medium supplemented with 10% fetal calf serum, penicillin (100 U/ml), and streptomycin (100 μg/ml). HSV type 2 strain 186 clone 3 isolated from a single plaque was used throughout this study (20). An aphidicolin-resistant mutant (Aph') was selected by serially passaging the wild type in cultures overlaid with aphidicolin-containing medium and was cloned three times before use. The virus stock was prepared in HEF by inoculating at a low multiplicity (0.01 PFU per cell) to avoid the formation of defective particles. For irradiation of HSV, stock suspensions were diluted 1:10 in phosphate-buffered saline, and a 1-ml volume in 60-mm dishes was exposed to a Toshiba GL-15 UV bulb for various time intervals at a dose rate of 20 ergs per mm² per s as measured by a Black-Ray UV dosimeter (model J-225). Essentials of the plaque assay procedures for HSV have been described elsewhere (18), and plaque numbers were counted with a dissecting microscope at ×10 magnification after staining. Aphidicolin was purchased from Wako Pure Chemicals.

Aphidicolin, a tetracyclic diterpene tetraol, is known to be a specific, direct inhibitor of the α-type DNA polymerase of eucaryotic organisms; unlike most other inhibitors of DNA synthesis, it does not affect the activities of DNA polymerases β and γ or the synthesis of RNA and protein (9, 11). In this study, we used this inhibitor to differentiate the activities of DNA polymerase α from those of other cellular DNA polymerases. Since the growth of HSV is sensitive to aphidicolin (21), we first tried to isolate Aph' of HSV, using aphidicolin as a selective agent. Plaque formation by the wild type was very sensitive to aphidicolin, and more than 90% of plaques were inhibited by 2 μg of aphidicolin per ml, whereas the plating efficiencies of Aph' were 100% at 1 μg/ml and 85% at 2 μg/ml (Fig. 1). Thus, Aph' of HSV can be used to determine whether DNA polymerase α is involved in HCR of UV-irradiated HSV.

The survival curves of UV-irradiated HSV in HEF, as well as those in various mammalian cells (16, 18), had two components at low virus concentrations, at which the multiplicity reactivation was negligible. The surviving fraction of Aph' irradiated with 6.0 × 10² ergs per mm² was usually about 0.0005, and at this concentration, multiplicity reactivation was not observed. Therefore, irradiation for 5 min at 20 ergs per mm² per s was adopted as the standard radiation dose through this study. Confluent monolayers of HEF were infected with UV (6 × 10³ ergs per mm²)-irradiated Aph'. After a 1-h adsorption period, cells were overlaid with 0.5% agarose in minimal essential medium containing 5% fetal calf serum and appropriate concentrations of aphidicolin, and the plaques were counted 3 days postinfection (p.i.). Plaque formation by UV-irradiated Aph' was reduced by more than 60% at a concentration of 1 μg of aphidicolin per ml, whereas this aphidicolin concentration did not affect the plating efficiency of nonirradiated Aph' (Fig. 2). These results indicate that an aphidicolin-sensitive cellular function was involved in the process of HCR of UV-irradiated HSV.

The next experiments were performed to determine time dependence of aphidicolin inhibition of plaque formation by UV-irradiated virus; the addition of aphidicolin at a time before the reactivation mechanism can act should inhibit plaque formation, whereas addition after the reactivation has occurred should have little inhibitory effect. Confluent monolayers of HEF were infected with UV-irradiated Aph', overlaid with 0.5% agarose in minimal essential medium containing 5% fetal calf serum, and incubated at 37°C, and at different times p.i. aphidicolin-containing agarose was further overlaid to make a final concentration of 1 μg/ml.

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Plaque formation by irradiated virus was still sensitive to aphidicolin at 12 h p.i., but it became entirely resistant by 36 h p.i. (Fig. 3).

Aphidicolin is a specific inhibitor of DNA polymerase α but not of DNA polymerases β and γ (9, 11). Furthermore, recent studies have shown that aphidicolin-resistant cell mutants have altered DNA polymerases which are resistant to aphidicolin (12, 25), indicating that DNA polymerase α is the true target of aphidicolin in vivo. The virus mutant used here was highly resistant to aphidicolin, and the plating efficiency of Aph⁻ was not affected by 1 μg of aphidicolin per ml. However, this concentration of aphidicolin markedly inhibited plaque formation by UV-irradiated Aph⁺ (Fig. 2) and suppressed cellular DNA synthesis by more than 80% (data not shown). Taken together, it is strongly suggested that DNA polymerase α plays an important role in the repair of UV-damaged HSV DNA. This conclusion might explain the previous findings that human cytomegalovirus infection enhances the reactivation of UV-irradiated HSV (18, 19), because human cytomegalovirus increases the activity of DNA polymerase α in infected cells (8).

On the other hand, we recently found that the repair synthesis of viral and cellular DNAs evoked at the late stage of HSV infection was entirely resistant to aphidicolin and phosphonoacetic acid, and it was suggested that DNA polymerase β was involved in repairing HSV-induced damage of viral and cellular DNAs which might be single-strand breaks (20a). These observations suggest that different types of damages of HSV DNA are repaired by different host cell DNA polymerases in vivo. Alternatively, both DNA polymerases α and β are indispensable for repair synthesis of UV-damaged viral DNA, and DNA polymerase β plays a major role in the repair of HSV-induced DNA damage at the late stage of infection. Our results seem to be in accord with those in the recent report by Miller and Chinault (14) in which, using permeable cells, they showed that DNA polymerases α and β participated differentially in DNA repair synthesis induced by different DNA-damaging agents.

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LITERATURE CITED

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