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EFFECT OF ULTRAVIOLET LIGHT ON DNA STRUCTURE IN 5-IODO-2′-DEOXYURIDINE-SUBSTITUTED HSV-1 DNA

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Abstract—Herpes simplex virus type-1 (HSV-1) was grown in the presence of 5-iodo-2′-deoxyuridine (IdUrd), and the virion-DNA was isolated by isopycnic centrifugation in CsCl. Irradiation of IdUrd-containing HSV DNA with either 302 nm or 254 nm ultraviolet (UV) light introduced strand breakage into the DNA in a dose-dependent manner when analyzed by alkaline sucrose density gradient sedimentation. Irradiation of unsubstituted HSV DNA under similar conditions produced little strand breakage. These observations are in agreement with the proposed mechanism for photochemical generation of strand breakage in 5-halo-2′-deoxyuridine-containing DNA. Irradiation of IdUrd-substituted virions followed by analysis of the isolated DNA indicated less strand breakage than irradiation of isolated IdUrd-substituted DNA under equivalent conditions. The dosage of irradiation required to introduce DNA strand breakage in IdUrd-substituted virions was equivalent to that employed to affect >99% loss of infectious virus activity in both control and IdUrd-containing virions. It is suggested that the relative UV insensitivity of IdUrd-substituted HSV may be due to the microstructure environment of the substituted HSV DNA which may favor recombination of the photochemically formed halogen-uracil radical pairs.

The incorporation of the halogenated pyrimidine deoxyribonucleosides, 5-bromo-2′-deoxyuridine (BrdUrd)§ or 5-iodo-2′-deoxyuridine (IdUrd), into the DNA of mammalian cells, bacteria or virus causes an increased sensitivity to ultraviolet (UV) irradiation [reviewed in Refs. 1 and 2]. The degree of UV sensitivity is dependent upon the wavelength employed and the sample environment [3–5]. A major exception to these observations was first reported by Wacker *et al.* [6] who found that BrdUrd incorporation into the DNA of herpes simplex virus sensitized the virus to UV irradiation as expected, but that IdUrd incorporation yielded protection.

Treatment of herpes simplex virus type-1 (HSV-1) infected cells with IdUrd leads to substitution of IdUrd for thymidine in the viral DNA with the degree of substitution parallel to the degree of inhibition of virus replication [7]. A recent study of the kinetics of UV inactivation of HSV-1 revealed an absence of UV sensitization of HSV by IdUrd substitution [8]. The present study examines the effect of UV irradiations on DNA structure in both control and IdUrd-substituted HSV-1 DNA.

MATERIALS AND METHODS

Cells and viruses. HSV-1 (F strain) was propagated in Vero cells at a multiplication of infection (MOI) of 0.01 plaque-forming units (pfu) per cell. Vero cells were grown as a monolayer culture in Dulbecco's minimum essential medium (DMEM) supplemented with 5% fetal bovine serum (FBS) and 5% newborn calf serum (GIBCO). For large scale virus growth, Vero cells were grown on Cytodex-1 beads (Pharmacia) in 850 cm^2 plastic roller bottles (Falcon) (1.5 g beads/roller bottle). Preparation of the beads and growth of the Vero cells were performed according to instructions of the manufacturer. When the Vero cells reached confluence they were infected with HSV-1 at a multiplicity of 10 pfu/cell. After a 1-hr adsorption period, the virus was removed, the cells were washed with phosphate-buffered saline (PBS), DMEM + 2% FBS were added, and the cultures were incubated at 37°. Three-and-one-half hours after the infection period the medium was removed and fresh DMEM + 2% FEBS were added. The drug-treated cultures contained 20 μM [^{125}I]IdUrd (sp. act. 40 μCi/μmol) and [^{32}P]sodium phosphate (400 μCi/mL) (Amersham), and the control cultures [2-^{14}C]dThd (0.5 μCi/mL) (Moravek). The cultures were incubated at 37° for 20 hr. Purification of control and IdUrd-substituted virions was performed according to Zucker and Prusoff [8]. Briefly, the cells were lysed by several cycles of freeze–thaw, beads and nuclei were removed by low-speed centrifugation, and the crude virus was pelleted. The resuspended "crude virus" was purified from cell debris by centrifugation through a linear 25–55% sucrose gradient, and the HSV-1 containing band removed and re-pelleted. This purified virion pellet was suspended in 3 mL of buffer (20 mM Tris–HCl, pH 7.5, 150 mM NaCl, 3 mM $MgCl_2$) and dialyzed against this buffer.

Infectious virus titer was determined by plaque assay as described by Lin *et al.* [9]. Virion particle number was determined spectrophotometrically according to Zucker and Prusoff [8].

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§ Abbreviations: BrdUrd, 5-bromo-2′-deoxyuridine; HSV-1, herpes simplex virus type-1; IdUrd, 5-iodo-2′-deoxyuridine; dThd, thymidine; and SDS, sodium dodecyl sulfate.

Isolation of control and IdUrd-substituted HSV-1 DNA. IdUrd-substituted or control HSV-1 virions were lysed by proteinase K-sarkosyl, and the liberated HSV-1 DNA was separated from cellular host DNA by isopycnic centrifugation on a CsCl gradient according to Fisher *et al.* [7]. Purified virions in an equal volume of a solution containing 20 mM Tris–HCl, pH 7.9, 10 mM EDTA, 2% sarkosyl (v/v) were treated with 350 μg/mL proteinase K at 37° for 2 hr. (At this stage all handling of DNA was carefully performed with pipettes with the tips cut off to avoid DNA shearing.) The reaction mixture was extracted with phenol-chloroform with gentle inversion, and then dialyzed at 4° against several changes of buffer (50 mM Tris–HCl, pH 7.4, 10 mM EDTA, 10 mM NaCl). The DNA solution was then added to buffered CsCl and the density adjusted to 1.77 g/mL for IdUrd-substituted HSV DNA, or 1.71 g/mL for control HSV DNA and centrifuged for 60 hr at 18° at 34,000 rpm in a 50Ti rotor. Approximately 80 fractions were collected from the bottom of the gradients, and the radioactivity and density (via refractive index) of every other fraction were determined. The fractions containing radioactivity-labeled HSV DNA were pooled and dialyzed against a TEN buffer (10 mM Tris–HCl, pH 7.4, 10 mM EDTA, 10 mM NaCl) at 4°.

Irradiation. Virion preparations were diluted to an equal particle concentration in phosphate-buffered saline, kept cold, and stirred during irradiation. HSV-1 DNA preparations were diluted in TEN buffer and were irradiated while cold without stirring (depth of solution < 1 mm). The ultraviolet light source was either a GE G-15T8 bulb (254 nm peak) or a UV Products 34-000-3901 bulb (302 peak; 284-400 nm distribution) with incident doses of 0.36 J/m^2/sec and 0.45 J m^2/sec respectively.

Sucrose gradient centrifugation. Irradiated virion preparations were diluted with 4 vol. of alkaline lysis buffer and warmed in a 60° waterbath for 5 min, then 10 min at room temperature, and then kept on ice for 20 min. Irradiated solutions of DNA were diluted with an equal volume of 1.5 N NaOH and kept on ice for 20 min. Samples (400 μL) were then layered on top of a 4.6 mL linear 2 to 20% alkaline sucrose gradient formed in an SW 50.1 polyallomer centrifuge tube and centrifuged at 30,000 rpm for 2 hr at 4°. Alkaline sucrose stock solutions contained 3 mM EDTA, pH adjusted to 12.5 (by addition of 2 M NaOH), and 1 M Na^+ (by the addition of 5 M NaCl). Alkaline lysis buffer contained, in addition to the above, 2% sarkosyl and 0.5% SDS (and no sucrose). Both solutions were prepared fresh prior to use. After centrifugation, samples were collected from the top of the gradient and collected in 3 drop fractions. Next, aliquots of 65 μL from each fraction were spotted on GF/C filter disks (Whatman) and batch washed three times with 5% trichloroacetic acid (TCA), once with ethanol, and then air dried; the amount of radioactivity was determined by liquid scintillation counting with Optifluor-O (Beckman) as scintillant.

RESULTS

To examine the mechanism by which IdUrd incorporation apparently protects HSV cultures from UV inactivation, the DNA isolated from irradiated IdUrd-substituted virions as well as the IdUrd-substituted HSV DNA which was irradiated after isolation from the non-irradiated IdUrd-substituted virions were examined. The effects of such UV irradiation can be measured directly by sedimentation of the DNA in alkaline sucrose gradients in which intact and broken single-strand molecules have different sedimentation velocities [10, 11]. Virions were grown in the presence of both [^{32}P]phosphate and [^{125}I]IdUrd (20 μM) in order to simultaneously radiolabel HSV DNA.

The results from the CsCl isopycnic centrifugation of IdUrd-substituted HSV DNA labeled with [^{125}I] and [^{32}P] are shown in Fig. 1. The IdUrd-substituted DNA sedimented at a density of 1.785 g/cc, whereas wild type HSV-1 has a density of 1.725 g/cc. The gram molecular weight of the DNA should increase 1.06397 times and density should increase to 1.835 if 100% of the dTMP residues are substituted with IdUMP. The observed buoyant density of the DNA (1.785 g/cc) corresponded to a 54% substitution of dTMP residues with IdUMP.

Previous study of UV inactivation of HSV virions demonstrated ≤1% survival of both control and IdUrd-substituted virions when exposed to an incident dose of 250 J/m^2 with either 302 nm or 254 nm incident light [8]. IdUrd-substituted virions were exposed to this dosage with a 302 nm incident light, and the DNA was isolated and analyzed by alkaline sucrose density gradient sedimentation (Fig. 2). DNA from irradiated virions sedimented 26.5 to 28.6% of the gradient (fractions 13–14 of 49 total fractions) while control DNA from non-irradiated virus sedimented 28.6 to 32.6% into the gradients (fractions 14–16). The absence of any large difference in sedimentation upon irradiation reflects only a slight introduction of single-strand breakage in the DNA. IdUrd-substituted virions irradiated with dosages of 302 nm light at ≤200 J/m^2 showed no difference in DNA sedimentation patterns (data not shown).

In contrast to the irradiation of whole virions, the irradiation of isolated IdUrd-substituted DNA at 302 nm resulted in an increased degree of DNA strand breakage. Irradiated IdUrd-DNA sedimented sharply 19% into the gradient (fraction 8 of 42 total fractions) versus control IdUrd-DNA which sedimented 26.2 to 28.6% (fractions 11–12) into the gradient. This represents an approximately 30% difference in sedimentation distance when IdUrd-DNA from irradiated virions is compared to direct inactivation of DNA. DNA strand breakage increased when the dose of irradiation was increased from 250 J/m^2 to 1000 J/m^2 (Fig. 3A). The same dose-dependent degree of strand breakage was seen with 254 nm incident light (Fig. 3B). There was only slightly less strand breakage seen with a dose of 250 J/m^2 of 302 nm light compared to the same dose at 254 nm light.

When DNA from isolated control virions was irradiated with either 302 nm or 254 nm light and subjected to alkaline sucrose density gradient analysis, only a slight difference in sedimentation was observed even at doses up to 1000 J/m^2 (Fig. 4).

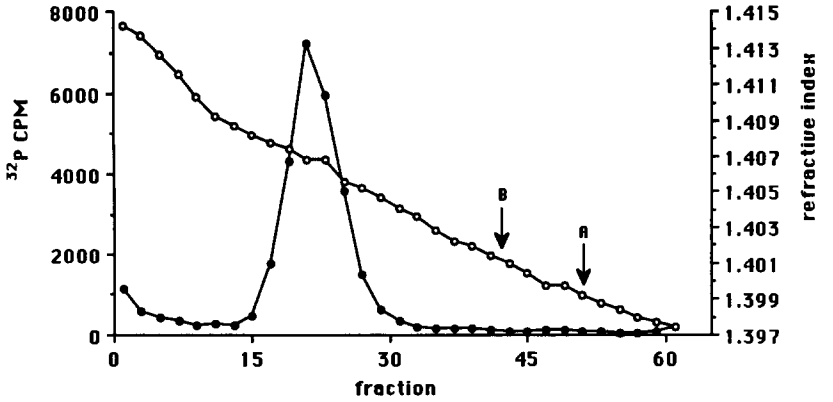

Fig. 1. Isopycnic centrifugation of IdUrd-substituted HSV-1 DNA in CsCl. DNA was isolated from HSV-1 virions grown in the presence of 20 μM [^{125}I]IdUrd and [^{32}P]sodium phosphate and sedimented in buffered CsCl as described in Materials and Methods. Direction of sedimentation is right to left. Key: (●) acid precipitable radioactivity [^{32}P]; and (○) refractive index. Arrows indicate the fractions in the gradient where unsubstituted host cell DNA (A) and unsubstituted HSV-1 DNA (B) sedimented. Host cell DNA has a refractive index of 1.3992 (1.699 g/mL); native HSV-1 DNA has a refractive index of 1.4017 (1.726 g/mL); and isolated IdUrd-substituted HSV DNA has a refractive index of 1.4070 (1.785 g/mL density). Acid precipitable radioactivity is shown as ^{32}P cpm; the ^{125}I radioactivity profile which is not shown coincided with the ^{32}P cpm profile.

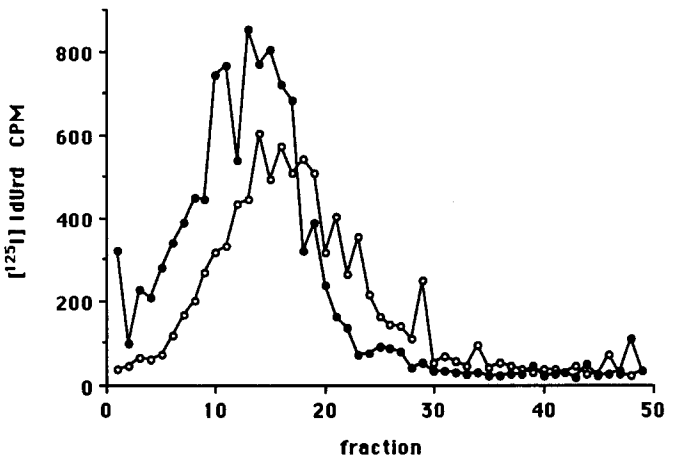

Fig. 2. Sedimentation in alkaline sucrose density gradient of irradiated [^{32}P]phosphate and [^{125}I]IdUrd-substituted HSV-1 virions. Virions were irradiated (302 nm light) at 2×10^8 pfu/mL, and DNA was isolated as described in Materials and Methods. Direction of sedimentation is left to right. Acid precipitable radioactivity [^{125}I]IdUrd: (●) 250 J/m^2; and (○) non-irradiated virions. Acid precipitable [^{125}I]IdUrd radioactivity is shown; the [^{32}P]phosphate radioactivity profile which is not shown coincided with the [^{125}I]IdUrd radioactivity profile.

Isolated unsubstituted HSV DNA then is not susceptible to significant UV-induced single-strand breakage at radiation doses that yield appreciable strand breakage in the corresponding IdUrd-substituted DNA.

These two findings are consistent with those usually observed for UV irradiation of native and 5-halo-2′-deoxyuridine containing DNA, i.e. when BrdUrd or IdUrd is incorporated into DNA the major lesion induced by UV light changes from pyrimidine dimer to single-strand breakage and alkali labile bond formation [2]. Mechanistically the initial photochemical event in 5-halo-2′-deoxyuridine-substituted DNA is photo-induced dehalogenation leaving a C-5 uracil free radical which can abstract an adjacent H atom on the C-2′ of the deoxyribose on the 5′ side of IdUrd. This sugar radical further rearranges to yield strand breakage or alkali-labile bond formation [1, 12, 13]. According to this mechanism, halogen loss from IdUrd-substituted DNA should parallel UV-induced strand breakage.

The overall sedimentation profiles involving [^{32}P]phosphate, [^{125}I]IdUrd-substituted DNA (i.e.

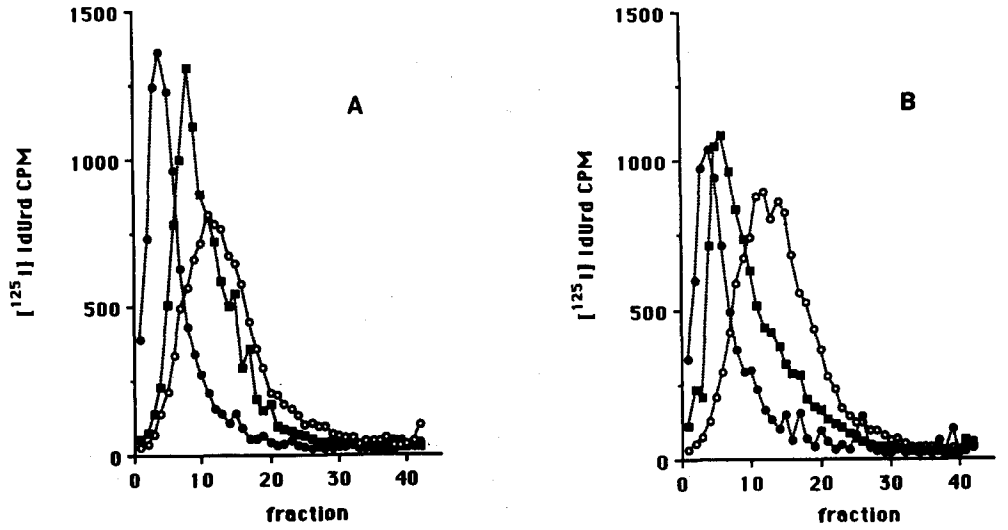

Fig. 3. Sedimentation in alkaline sucrose density gradient of irradiated [^{32}P]phosphate-labeled [^{125}I]IdUrd-substituted HSV DNA. HSV DNA substituted with [^{32}P]phosphate and [^{125}I]IdUrd was isolated and irradiated at a concentration of 10 mg/mL. A 2-μg sample was subjected to centrifugation as described in Materials and Methods. The dose of radiation was: (●) 1000 J/m^2; (■) 250 J/m^2; and (○) none. Direction of sedimentation is left to right. Panel A: 302 nm light; panel B: 254 nm light. Acid precipitable [^{125}I]IdUrd radioactivity is shown; the [^{32}P]phosphate radioactivity profile coincided with the [^{125}I]IdUrd radioactivity profile and is not shown.

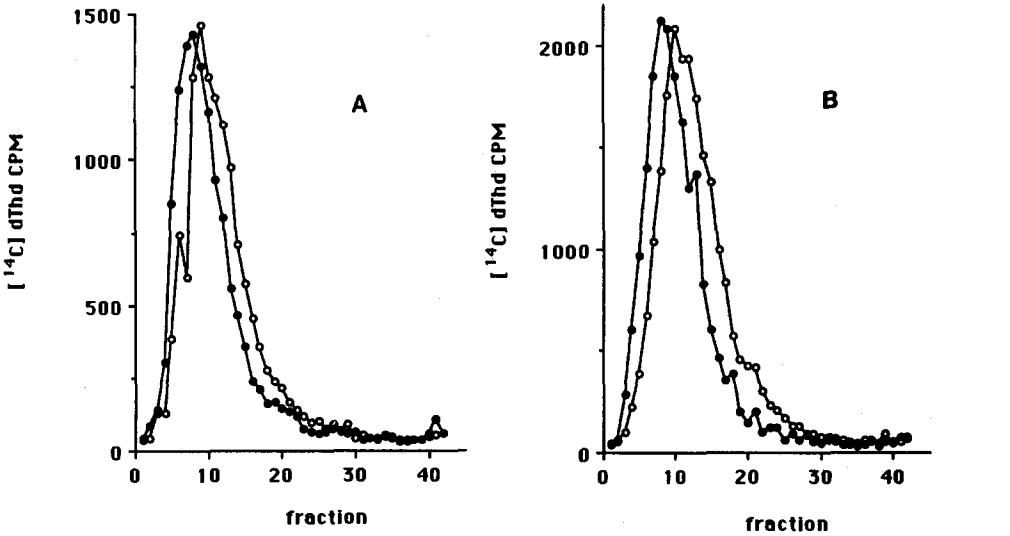

Fig. 4. Sedimentation in alkaline sucrose density gradient of irradiated [^{14}C]dThd HSV DNA. [^{14}C]dThd DNA was irradiated at 10 μg/mL with a 2-μg sample subjected to centrifugation as described in Materials and Methods. Direction of sedimentation is left to right. The dose of radiation was: (●) 1000 J/m^2; and (○) non-irradiated sample. Panel A: 302 nm light; panel B; 254 nm light.

Figs. 2 and 3) were identical whether ^{32}P or ^{125}I radioactivity (as shown) was plotted per fraction. However, the ^{32}P/^{125}I ratio in the DNA substituted with IdUrd was altered upon UV irradiation. For example, at the peak sedimentation fraction the ^{32}P/^{125}I ratio in a non-irradiated IdUrd-substituted HSV DNA was 1.50. As shown in Fig. 5, upon irradiation of this DNA with 1000 J/m^2 of 302 nm light, the ^{32}P/^{125}I ratio at the peak sedimentation fraction increased to 1.83. Similarly, irradiation of IdUrd-substituted DNA with 254 nm light produced an increase in the ^{32}P/^{125}I ratio of the peak sedimentation fraction from 0.536 in a non-irradiated DNA to 0.625 after a 1000 J/m^2 dosage at this wave-

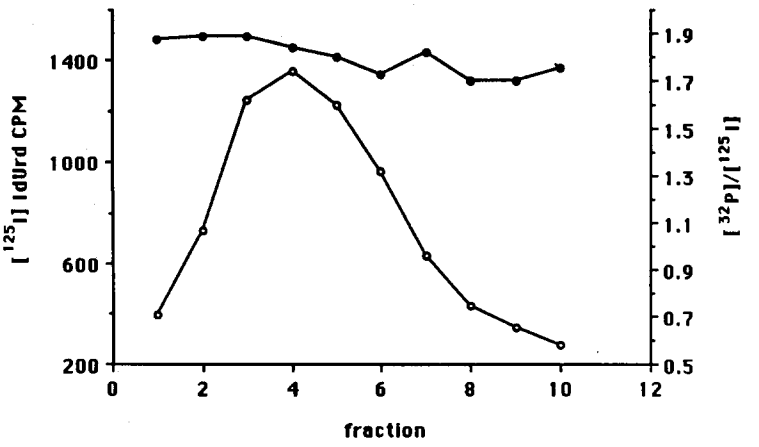

Fig. 5. Sedimentation in alkaline sucrose density gradient of [^{32}P]sodium phosphate and [^{125}I]IdUrd-substituted HSV DNA indicating ^{32}P/^{125}I ratios. DNA was irradiated with 1000 J/m^2, 302 nm light. Direction of sedimentation is left to right. Key: (○) [^{125}I]acid precipitable radioactivity; and (●) ^{32}P/^{125}I cpm ratio. Acid precipitable [^{125}I]IdUrd radioactivity is shown; the [^{32}P]phosphate radioactivity profile which is not shown coincided with the [^{125}I]IdUrd radioactivity profile.

length. It is apparent that the UV irradiation of IdUrd-substituted HSV DNA effected a halogen loss with a concomitant strand breakage. This observed halogen loss was about 18% with 1000 J/m^2 of 302 nm light and about 14% with 1000 J/m^2 of 254 nm light.

DISCUSSION

HSV DNA containing 54% substitution of IdUrd for dThd residues undergoes a photochemically induced lesion when analyzed by alkaline sucrose density gradient centrifugation, leading to alkali labile bond formation and strand breakage. This damage occurred in a dose-dependent manner at either 302 or 254 nm light and was associated with an observable loss of iodine from the substituted DNA. This observable damage to IdUrd-DNA was less when the irradiation occurred on the intact virion than on isolated IdUrd-DNA. A dosage of 250 J/m^2 of 302 nm light caused observable strand breakage in isolated IdUrd-DNA (Fig. 3A), but less change in DNA sedimentation velocity from irradiated IdUrd virions (Fig. 2). This dosage, however, was enough to cause a >99% drop in infectious virus activity in both control and IdUrd virions. At dosages of ≤200 J/m^2 (302 nm) no IdUrd-DNA damage was detected, while considerable loss of infectious virus activity has been observed [8].

Zucker and Prusoff [8] investigated some biochemical consequences of UV irradiation of control and IdUrd HSV and reported that IdUrd substitution did not alter either levels of nuclease-resistant photoproducts, or the ability of irradiated virions to express the delayed early viral enzymes thymidine kinase and DNA polymerase, or the cellular repair of UV light induced lesions.

The similarity of these biochemical processes reflects the absence of any UV sensitization by IdUrd substitution in HSV DNA. In the present report UV light induced DNA strand breakage in IdUrd-substituted DNA was observed only at radiation dosages in excess of that required to affect considerable lowering of infectious virus activity. This absence of UV sensitization may be due to the fact that doses of UV light required to inhibit viral replications are far below those required to generate significant photochemical dehalogenation leading to strand breakage. This implies that the rate of photochemically-induced strand breakage is operating on a much reduced scale in IdUrd-substituted HSV DNA relative to other systems where IdUrd incorporation into DNA increases UV sensitivity [4, 5, 14].

An insight into this phenomenon comes from the photochemical studies of Murry and Martin [15] using sequencing gels to analyze UV damage to IdUrd or BrdUrd containing DNA of known sequences. They found that the degree of UV damage varied considerably, with nearly complete cleavage at sites in which IdUrd or BrdUrd substituted for thymidine in the consensus sequence CTT. No UV damage was detected at sites in which IdUrd or BrdUrd substituted for thymidine in the consensus sequence GTR where R is a purine nucleoside. These workers concluded that the microstructure of DNA, in particular the distance between the UV formed free radical at carbon 5 of the pyrimidine base and the hydrogen on the 2′-carbon of the adjacent deoxyribose on the 5′-side, ultimately determines the degree of cleavage. The larger the distance between these two carbons the smaller the degree of damage, and conversely the smaller the distance the larger the degree of damage. Whether the microstructure environment about a significant number of IdUrd substitutions for thymidine residues in HSV DNA favors recombination of photochemically formed uracil–halogen radical pairs, or whether the incorporation of IdUrd into HSV DNA itself causes a

change in this microstructure environment at the site of the analog remains to be investigated. In addition, HSV-1 has a high deoxyguanosine content [16], there is a high probability that IdUrd is incorporated adjacent to a deoxyguanosine, thereby generating a site of minimal potential damage.

Likewise, the structure of the HSV virion itself may play an important role in this process. HSV-1 virions are composed of an electron-opaque core, an icosahedral capsid, an electron-dense tegument, and a spiked envelope (reviewed in Ref. 16). At least six virion polypeptides are closely associated with the viral DNA and may affect the microstructure environment at the sites of IdUrd incorporation either directly or by alteration in their configuration and thus retard the rate of photoproduct formation in the intact virion.

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