Inactivation by U.v.-irradiation of Interfering Herpes Simplex Virus Particles: Interference Requires a Functional Genome

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SUMMARY

The sensitivity of interfering herpes simplex virus (HSV) particles to u.v.-irradiation was studied in a virus stock of HSV-1 strain ANG that contained an excess of interfering over infectious particles. Following u.v.-irradiation, samples of this virus stock were assayed for their plaque-forming capacity and their capacity to interfere with the replication of unirradiated standard virus. Depending on the assay conditions, interfering particles appeared to be less, equally, or more sensitive to u.v. light than infectious particles. It is concluded that interference is a gene function of interfering particles rather than being exerted directly by structural constituents of these particles.

Virus particles containing only a fraction of the genetic information of standard virus (D particles) and particles interfering with the replication of progeny virus (I particles) have been identified in stocks of herpes viruses obtained after successive passages at a high m.o.i. (Bronson et al. 1973; Ben-Porat et al. 1974; Wagner et al. 1974; Fleckenstein et al. 1975; Frenkel et al. 1975; Murray et al. 1975; Schröder et al. 1975/76; Campbell et al. 1976). In one case herpes D particles could be correlated to interference (Murray et al. 1975) as has been shown for a number of animal viruses (Huang, 1973). At present, neither D particles nor I particles derived from herpes viruses can be physically separated from infectious virus particles.

The mechanism by which I particles reduce the formation of infectious progeny virus remains to be elucidated. Previously, we reported that I particles in virus stocks of herpes simplex type I (HSV-I) strain ANG (Schröder et al. 1975/76; Darai & Munk, 1976) may exceed infectious particles by one order of magnitude (Schröder & Urbaczka, 1978). Furthermore it could be demonstrated that I particle–cell interaction does not necessarily lead to cell death. In order to understand the intracellular events following the interaction of cells with I particles and with infectious particles it is necessary to establish whether interference requires the expression of gene functions of I particles. Alternatively interference could be exerted directly by structural constituents of I particles. Regulatory active constituents of HSV particles have recently been reported to suppress the synthesis of cellular macromolecules (Fenwick & Walker, 1978). In this report we present evidence that interference in HSV-1 strain ANG requires the expression of one or more functions specified by the genome of I particles.

The u.v.-inactivation of I particles was studied in virus stock HP18 of HSV-1 strain ANG, which has been reported to contain a 16-fold excess of I particles over infectious particles (Schröder & Urbaczka, 1978). Virus was exposed to u.v.-light at 80 ergs/mm²/min. During irradiation time of 2 to 6 min, samples (25 ml in open 145 mm Petri dishes) were kept at 0 °C. The presence of infectious and of I particles within one stock allowed us to relate the u.v. inactivation of I particles to that of infectious particles.

First the u.v.-damage to I particles was determined by co-infection of RC-37 cultures with unirradiated standard virus at a constant high m.o.i. of 5 and with HP18 at various m.o.i. either untreated or u.v.-irradiated. When co-infections were carried out with untreated
Non-irradiated HP18 virus (p.f.u./cell)

Fig. 1. Interference in RC-37 cells infected with five infectious standard virus particles/cell. Confluent cultures of $1.5 \times 10^6$ African green monkey kidney cells, RC-37 (Italdiagnostics, Rome, Italy), per plastic Petri dish (60 mm) were used. One series of cultures was co-infected with standard virus, LP5 (Stegmann et al. 1978), at a constant m.o.i. of 5 and with unirradiated HP18 at varying m.o.i. ($\bullet$). In two additional series of cultures, u.v.-irradiated samples of HP18 virus were used for co-infection, one sample was u.v.-inactivated by a factor of 75 ($\square$) and another by a factor of 140 ($\bigotimes$), as judged by the reduction of their plaque-forming capacities on RC-37 cultures (Russell, 1962). For the irradiated samples volume equivalents corresponding to the indicated m.o.i. of unirradiated HP18 were applied. The conditions for adsorption and formation of progeny virus have been described elsewhere (Schröder & Urbaczka, 1978). The production of progeny virus was terminated by two cycles of freezing and thawing 24 h p.i. Low speed supernatants of the lysates (5 ml) were assayed for plaque-forming virus on RC-37 cell cultures. Progeny virus formation is given as percent of the infectious titre (1.9 × 10⁶/ml) observed for a culture infected with standard virus exclusively. The data are the means of two independent experiments.

HP18 at increasing m.o.i. (from 0.05 to 5.0), formation of progeny virus decreased proportionally. The yields of infectious progeny virus in individual assays plotted versus the m.o.i. of HP18 in a log-log scale fitted a straight line (Fig. 1). Co-infections with two u.v.-irradiated samples of stock HP18 were performed using volume equivalents representing up to five times the volume of untreated stock HP18 when applied at a m.o.i. of 5. The yields of infectious progeny virus obtained fitted two separate lines parallel to that obtained for co-infection with untreated HP18 (Fig. 1). The individual distances on the abscissa to the latter line indicate factors of 77 and 145 by which the interfering capacity of the HP18 samples has been reduced due to u.v.-irradiation. The plaque-forming capacity on RC-37 cultures of the two irradiated HP18 samples were reduced by factors of 88 and 140 respectively. Taken together, these data demonstrate that under assay conditions of co-infection with standard virus at a m.o.i. of 5 (high m.o.i. co-infection), I particles appear to be as sensitive to u.v. light as infectious particles. As a consequence the relative interfering activity of stock HP18 expressed as interfering capacity per plaque-forming unit remained unaffected by u.v. irradiation.

Similar co-infection experiments were performed with standard virus at a constant m.o.i. of 0.2 (low m.o.i. co-infection). Under these conditions the relative interfering activity of the same u.v.-irradiated HP18 samples that were tested above in high m.o.i. co-infections was apparently increased by a factor of about 12. In 16 independent experiments using 8 samples of stock HP18 inactivated 20- to 412-fold by u.v.-irradiation with regard to their plaque-forming capacity, a mean increase in the relative interfering activity by a factor of 16 was observed. The alteration of the relative interfering activity was not dependent on the degree of u.v.-damage. A determination of the interfering capacity following u.v.-irradiation
Fig. 2. Interference in RC-37 cells infected with one infectious standard virus particle/cell. (a) One series of cultures was co-infected with standard virus at a constant m.o.i. of 0.2 and with non-irradiated HP18 at varying m.o.i. (●—●). In another series, cells were co-infected with a u.v.-irradiated sample of HP18, the infectious capacity of which was reduced by a factor of 31 (○—○). In (b), infection with unirradiated (●—●) and irradiated HP18 (○—○) is preceded by standard virus infection for 2 h. As in Fig. 1, the indicated m.o.i. applies only for unirradiated virus while in the case of irradiated virus, volume equivalents were used; 100% of infectious progeny virus corresponds to the infectious titre (4 × 10^7/ml) of a culture infected with standard virus exclusively. Further experimental details are given in the legend to Fig. 1.

when tested upon low m.o.i. co-infection is given in Fig. 2(a) for a sample of HP18 which had been reduced by a factor of 31 in its plaque-forming capacity. The reduction of the interfering capacity was determined to be 2.1. Hence the relative interfering activity was increased by a factor of 15.

In low m.o.i. co-infections, re-cycling of infectious virus produced may occur but does not appear to influence greatly the experimental data. The virus yield (intracellular plus extracellular) of a culture where one fifth of the cells had been infected with standard virus was one fifth of the yield observed in cultures in which all of the cells had been infected (see legends to Fig. 1 and 2). Furthermore, comparisons of virus samples with respect to their interfering capacities are based on comparisons of dilutions of these samples causing the same degree of interference.

Possibly the lower sensitivity of I particles to u.v.-light observed upon low m.o.i. co-infection (Fig. 2a) as compared to high m.o.i. co-infection (Fig. 1) is due to a preferential repair of I particles under conditions where only one infectious particle and one or more interfering particles per cell are involved. In co-infections at high m.o.i. where five infectious particles and one or more I particles per cell are involved, repair of the u.v.-damaged DNA of I particles might be reduced. One argument for this hypothesis might be the acceleration of the infectious cycle due to a gene dose effect when cells are infected at high m.o.i. If so, the apparent increase of the relative interfering activity of stock HP18 following u.v.-irradiation when tested via low m.o.i. co-infections should be reversed when standard virus infection of cells at low m.o.i. is allowed to proceed for some time before the cells are superinfected with u.v.-irradiated stock HP18.

Fig. 2(b) shows the results of experiments performed in order to test the latter assumption. Cultures were infected with standard virus at a m.o.i. of 0.2 and superinfected with various dilutions of u.v.-irradiated HP18 2 h later. The u.v.-irradiated sample of HP18 was the same as that used in the experiment of Fig. 2(a). In control experiments, unirradiated HP18 was used for superinfection. A comparison of Fig. 2(a) and (b) reveals that I particles appear to be much more sensitive to u.v.-light when the u.v.-damage was assayed under the conditions of the experiment of Fig. 2(b) than under those of Fig. 2(a).
While the relative interfering activity of the same u.v.-irradiated HP18 sample appeared to be increased 15-fold when tested via low m.o.i. co-infection (Fig. 2a) it was decreased by a factor of about two when tested via subsequent infections (Fig. 2b). Apparently early standard virus infection allowed less 'repair' of the u.v.-damage inflicted on I particles. For a hypothetical constituent of I particles which interferes directly, one should observe the same apparent sensitivity to u.v.-light in both types of experiments. Therefore we conclude that interference is a gene function of I particles rather than being exerted directly by the infecting I particles themselves. The presence of functional nucleic acid in I particles has been postulated to be a general criterion for homologous interference by I particles of animal viruses (Huang, 1973).

Combining u.v.-irradiated I particles present in stock HP18 and non-irradiated standard virus within one cell, a procedure necessary in order to determine the apparent sensitivity to u.v.-light of I particles, obviously complicates the above experiments. Factors influencing the biological activity of u.v.-irradiated I particles could be provided by the unirradiated infectious virus particles and/or by cellular DNA repair systems.

In order to assess the contribution of cellular DNA repair we assayed the interfering activity of u.v.-irradiated I particles in u.v.-excision repair deficient Xeroderma pigmentosum cells of complementation group A (Cleaver & Bootsma, 1975), CRL 1223 (ATCC, Rockville, Md., U.S.A.). Again, the u.v.-irradiated sample of HP18 was the same one that had been used in the experiments shown in Fig. 2. Its interfering capacity was compared with unirradiated HP18 in a similar set of low m.o.i. co-infections as shown in Fig. 2(a). The apparent interfering capacity of the u.v.-irradiated HP18 sample on CRL 1223 cells was found to be reduced by a factor of 10 as compared to 2-1 when tested on RC-37 cells (Fig. 2a). On the other hand the relative interfering activity of the u.v.-irradiated HP18 sample on CRL 1223 cells was increased dramatically due to a more than $2 \times 10^4$-fold reduction of its plaque-forming capacity on CRL 1223 cultures as compared to the 31-fold reduction on RC-37 cultures. (The plaque-forming capacity of unirradiated virus on CRL 1223 cultures was determined to be 65% of that observed on RC-37 cultures.) The calculated value for the relative interfering activity of the u.v.-irradiated stock HP18 thus increased at least by a factor of $2 \times 10^4$ when u.v.-inactivation both of infectious and of I particles was determined with CRL 1223 cultures as compared to the 15-fold increase observed with RC-37 cultures.

The higher apparent sensitivity to u.v.-light of infectious virus particles in a plaque-assay using CRL 1223 cells as compared to DNA repair-competent cells confirm data already reported by other laboratories (Rabson et al. 1969; Lytle et al. 1972). The extreme increase of the relative interfering activity of stock HP18 on CRL 1223 cells following u.v.-irradiation remains to be explained. Possibly unirradiated standard virus present in interference assays provides functions facilitating the expression of u.v.-damaged genomes of I particles. It could be that in contrast to cellular DNA polymerases, HSV-specified DNA polymerase acts readily on u.v.-irradiated templates thus multiplying the DNA copies available for transcription.

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REFERENCES


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