Effect of Ultraviolet Irradiation on Infectivity and Deoxythymidine Kinase Activity of Herpes Simplex Viruses[1] (36237)

TAKEO OGINO AND FRED RAPP

Department of Microbiology, College of Medicine, The Milton S. Hershey Medical Center of The Pennsylvania State University, Hershey, Pennsylvania 17033

Various viral functions are inactivated to a different extent by ultraviolet (UV) irradiation. This has been used for the purpose of determining the target sizes of the genome of DNA viruses (1–5).

Recently, UV-irradiated herpes simplex virus (HSV) type 2 has been shown to transform hamster embryo fibroblast cells *in vitro* (6, 7), suggesting that UV-irradiated non-plaque-forming virus particles still retain sufficient information to transform mammalian cells. It has also been reported that UV-irradiated HSV type 1 can transform the deoxythymidine (TdR) kinaseless mouse L cells to a TdR kinase positive phenotype (8).

TdR kinase activity has been reported to increase in cells infected with HSV and some of these studies suggest that TdR kinase in HSV-infected cells is specified by the viral genome (9, 10). In order to relate loss of infectivity with loss of ability to induce the enzyme, we decided to examine the effect of UV-irradiation on the TdR kinase-inducing capacity of HSV types 1 and 2.

Materials and Methods. Cells. Monolayer cultures of primary rabbit kidney (RK) and primary hamster embryo fibroblast (HEF) cells were prepared as previously described (11, 12). The medium used for cell cultivation consisted of Eagle's basal medium with 10% fetal calf serum, 10% tryptose phosphate broth and 0.075% $NaHCO_3$ (closed cultures) or 0.23% $NaHCO_3$ (open cultures). All media contained 100 units of penicillin and 100 μg of streptomycin/ml.

Virus. Herpes simplex virus (HSV) strains used in this study were Seibert (type 1) and 316D (type 2), originally obtained from Dr. William Rawls, Baylor College of Medicine, Houston, Texas. Virus stocks were prepared in primary RK cells in 8 oz bottles as previously described (13). Infectively of virus was titrated by the plaque assay in RK cells under a 0.5% methylcellulose overlay (11).

UV-irradiation of virus. Undiluted virus (1.5 or 2.5 ml) was placed in a 60 × 15 mm Pyrex petri dish and irradiated at a distance of 15 cm from the UV source (GE, G8T5 UV bulb) for various times at room temperature. The solution was shaken constantly during the exposure of the virus.

Infection of cells. Confluent monolayer cultures in 8 oz bottles were washed once with 0.025 *M* Tris (hydroxymethyl)aminomethane (Tris) buffered saline (Tris buffered saline, pH 7.4) and inoculated with 1.0 ml of virus suspension at the indicated multiplicity after adsorption for 1 hr at room temperature. The cells were washed once with Tris buffered saline and covered with 20 ml of Eagle's basal medium supplemented with 2% fetal calf serum; 0.075% $NaHCO_3$; 100 units/ml of penicillin; and 100 μg/ml of streptomycin.

Enzyme assay. At designated times, the culture medium was removed; and cells were washed three times with Tris buffered saline (pH 7.4), scraped loose with the aid of a rubber policeman, and centrifuged. They were then suspended in approximately 0.5 ml of 0.15 *M* KCl in 0.05 *M* Tris buffer (pH 8.0) containing 0.003 *M* 2-mercaptoethanol. The suspensions were subjected to ultrasonic disintegration. The sonicated preparations were centrifuged at 15,000 rpm for 60 min in the SS-34 rotor of a Sorvall centrifuge. The

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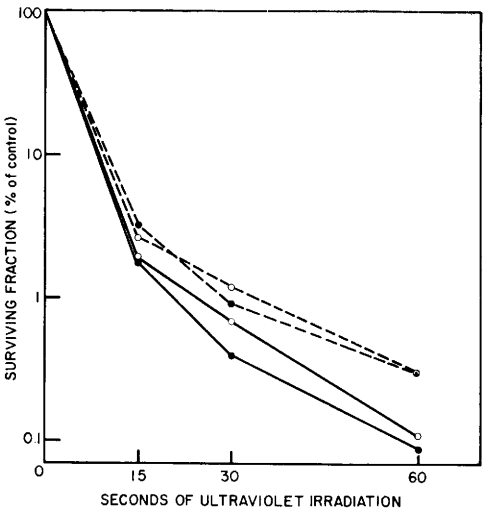

FIG. 1. Survival curves for irradiated herpes simplex virus type 1 (○); and type 2 (●) in RK cells; (—) 1.5 ml; or (- -) 2.5 ml in 60 mm Pyrex petri dishes were irradiated as described in Materials and Methods.

supernatant fractions were used as the source of enzyme for the assay of TdR kinase activity. Protein determinations of cell extracts were carried out by the method of Lowry *et al.* (14) using bovine serum albumin as the reference standard.

The activity of the cellular extract was assayed as previously described (15) in a mixture consisting of: TdR–CH_3-^{14}C, 0.1 or 0.2 μCi (54.7 μCi/μmole); ATP, 5 m*M*; $MgCl_2$, 5 m*M*; cellular extracts, approximately 50–250 μg of protein; 0.05 *M* Tris buffer (pH 8.0), up to 0.25 ml. In most assays, cold TdR (10 nmoles) was added. The reaction was conducted at 38° for 15 min and terminated by immersing the tubes into a boiling water bath for 2 min. The coagulated protein was removed by low speed centrifugation and the amount of TdR nucleotides in a 25 μl aliquot was assayed by the DEAE-cellulose disc method. Under these conditions, the enzyme activity was linearly related to both the amount of cellular protein and to the time of incubation. Specific activity of the enzyme is defined as the millimicromoles of TdR nucleotide produced in the reaction per 15 min/mg of protein in the cellular extracts under the described condition.

Results. UV-inactivation of infectivity. Survival curves for both HSV types 1 and 2 to UV-irradiation always had two component

TABLE I. TdR Kinase Activity in RK Cells Infected with UV-Irradiated HSV Types 1 or 2.

	Type:	1 (Seibert)		2 (316D)	
Expt. no.	UV-irradiation[a] (sec)	TdR kinase activity[b]	Infectivity (% of non-irradiated virus)	TdR kinase activity[b]	Infectivity (% of non-irradiated virus)
1	0	61.6	100 (5)[c]	34.6	100 (2)
	15	27.1	2.7	20.0	3.2
	30	18.0	1.2	5.2	0.9
	60	4.0	0.3	1.7	0.3
	Uninfected	0.12	0	0.19	0
2	0	73.6	100 (6.4)	61.6	100 (0.55)
	15	54.0	2.3	2.5	2.4
	30	11.2	1.3	1.2	1.1
	60	0.9	0.3	0.9	0.2
	Uninfected	0.88	0	0.57	0

[a] Virus solution (2.5 ml) in 60 mm Pyrex petri dishes were irradiated as described in Materials and Methods.

[b] ^{14}C-TdR phosphorylated (nmoles/mg of protein/15 min) at 38°; assayed 16 hr postinoculation of virus.

[c] Values in parentheses show the input multiplicity of nonirradiated virus as PFU/cell.

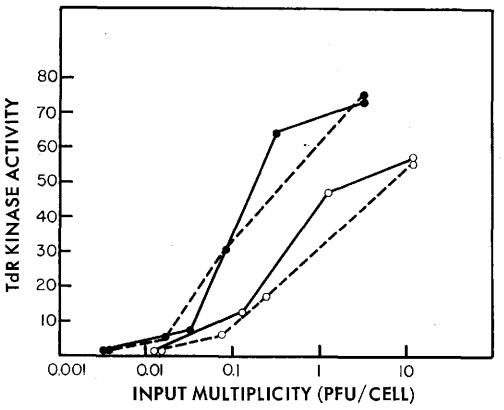

FIG. 2. TdR kinase activity in RK cells infected with various input multiplicities of nonirradiated virus and UV-irradiated virus: The input multiplicities of UV-irradiated virus were calculated by multiplying the input multiplicity of nonirradiated virus solution by the amount of inactivation. Virus solution (1.5 ml) was irradiated under the conditions described in Materials and Methods. TdR kinase activities at 16 hr postinoculation are shown (nmoles of ^{14}C-TdR phosphorylated/mg of protein/15 min) at 38°. Input multiplicity is shown as PFU/cell: (○—) type 1, nonirradiated; (○ - -) type 1, irradiated; (●—) type 2, nonirradiated; and (● - -) type 2, irradiated.

characteristics as shown in Fig. 1. Inactivation rate was almost the same for both types of viruses, although in most experiments, type 2 was a little more sensitive to UV-inactivation than type 1. Since crude virus solutions and different concentrations of virus were used, we cannot conclude that type 2 is inactivated at a faster rate than type 1, as other studies have suggested (7, 16). The nonlinear inactivation of HSV has recently been ascribed to host cell reactivation (17). Most of the plaques produced following UV-irradiation of virus were smaller and less clear than those induced by nonirradiated virus.

Effect of UV-irradiation on ability of HSV to induce TdR kinase activity. Increasing doses of UV-irradiation progressively inactivated the TdR kinase activity-inducing capacity in RK cells of both types of viruses (Table I). There is a possibility that the decrease of TdR kinase activity following UV-irradiation is not due to the loss of inducing capacity, but to a delay in expression. The results in Fig. 2 show that TdR kinase activity in cells infected 16 hr previously with UV-irradiated or nonirradiated HSV

TABLE II. TdR Kinase Activity in HEF Cells Infected with UV-Irradiated HSV Types 1 or 2.

	Type:	1 (Seibert)		2 (316D)	
Expt. no.	UV-irradiation[a] (sec)	TdR kinase activity[b]	Infectivity (% of non-irradiated virus)	TdR kinase activity	Infectivity (% of non-irradiated virus)
1[d]	0	64.8	100 (11)[c]	43.2	100 (1.1)
	15	23.0	2.2	2.8	1.2
	30	17.6	0.56	0.55	0.2
	60	6.5	0.08	0.90	0.04
	Uninfected	0.45	0	0.37	0
2[e]	0	42.7	100 (12.6)	13.1	100 (4.4)
	15	23.5	1.9	9.1	1.8
	30	18.9	0.69	2.6	0.38
	60	8.2	0.11	1.5	0.09
	Uninfected	0.44	0		

[a] Virus solution (1.5 ml) in a 60 mm Pyrex petri dish were irradiated as described in Materials and Methods.

[b] ^{14}C-TdR phosphorylated (nmoles/mg of protein/15 min) at 38°.

[c] Values in parentheses show the input multiplicities of nonirradiated virus (PFU/cell).

[d] TdR kinase activities were assayed at: 18 hr postinoculation (Expt. 1); and [e] 16 hr postinoculation (Expt. 2).

TABLE III. TdR Kinase Activity in HEF Cells Infected with Various Multiplicities of Nonirradiated HSV Types 1 or 2.

Type 1 (Seibert)		Type 2 (316D)	
Input multiplicity (PFU/cell)	TdR kinase activity[a] 18 hr PI[b]	Input multiplicity (PFU/cell)	TdR kinase activity[a] 18 hr PI[b]
11	53.5	1.1	62.7
1.1	44.2	0.11	25.7
0.11	20.0	0.011	1.9
0.011	3.4	0.0056	0.69
0.0011	0.61	0.0011	0.54
Uninfected	0.45	Uninfected	0.37

[a] (nmoles ^{14}C-TdR phosphorylated/mg protein/15 min) at 38°.

[b] Postinfection.

corresponds to infectivity of the virus preparations. Similar results were obtained 24 hr postinoculation. Results obtained in HEF cells with UV-irradiated virus (Table II) and with various multiplicities of nonirradiated virus (Table III) were similar to those observed with the RK cells.

Discussion. The effect of UV-irradiation on TdR kinase activity-inducing capacity has been examined with cells infected with DNA viruses (18–21). UV-irradiated rabbit poxvirus induced TdR kinase activity after inactivation of 5 logs of virus (18). UV-irradiated vaccinia virus also induced almost the same levels of TdR kinase activity as normal virus following destruction of 4 logs of virus (19, 20). These data suggest that TdR kinase activity-inducing capacity of a poxvirus is much more resistant to UV-irradiation than inactivation of infectivity.

In the present study, the ability to induce TdR kinase in RK and HEF cells with HSV types 1 and 2 was inactivated progressively by increasing doses of UV-irradiation. This sensitivity is similar to that reported with simian papovavirus SV40 (21). As shown in Fig. 2, the pattern of TdR kinase activity in RK cells infected with UV-irradiated virus is similar to that of normal virus corresponding to infectivity of survival virus. These observations suggest that capacity to induce TdR kinase activity and infectivity of HSV types 1 and 2 have similar sensitivities to UV-irradiation.

Summary. Inactivation of HSV types 1 and 2 by UV-irradiation was nonlinear and exhibited a two-component effect. UV-irradiated virus produced smaller and less clear plaques than normal virus. Ability of HSV to induce TdR kinase activity was progressively inactivated by increasing doses of UV-irradiation at a rate similar to inactivation of the infectivity of the virus.

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