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Effect of UV Light on RNA-Directed DNA Polymerase Activity of Murine Oncornaviruses

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The UV inactivation of RNA-directed DNA polymerase activity of Rauscher leukemia virus was shown to be due to damage to the protein. The UV dose resulting in 37% survival of viral polymerase activity at 254 nm was 2.4×10^4 to 3.1×10^4 ergs/mm^2. The inactivation rate of p30, a major internal viral protein, was much slower.

In a previous paper (8) it was reported that UV irradiation (400 ergs per mm^2 per s for 1 h) of Rauscher leukemia virus resulted in approximately 99% loss of endogenous polymerase (reverse transcriptase) activity. The irradiated virus produces neither provirus DNA nor progeny (5, 8). Since reverse transcriptase activity is essential for viral infectivity (3), quantitative studies of susceptibility to UV light inactivation were made.

Rauscher leukemia virus was harvested from the chronically infected JLS V-9 cell line (13), freed of cellular debris by low-speed centrifugation and frozen at −70 C until use. Ten milliliters of the clarified virus suspension was layered over two density zones of sucrose (0.5 ml of 50% sucrose and 1.5 ml of 10% sucrose), and the virus was sedimented to the interface between zones by centrifugation for 30 min at 39,000 rpm in a Spinco SW41 rotor. Virus suspension (0.5 ml) was diluted 1:4 with 0.01 M Tris buffer (pH 7.4), containing 1 mM EDTA. The virus was irradiated with a General Electric germicidal lamp (G15T8) in open, 50-mm plastic dishes on ice with agitation. More than 85% of its radiation is emitted at 254 nm (6). An intensity of 40 ergs per mm^2 per s was measured at the surface of the virus suspension by an UV intensity meter with a selenium cell (Ultraviolet Products, San Gabriel, Calif.). Samples were taken for assay at various intervals.

The infectivity titer as measured by the XC plaque assay (12) dropped rapidly with a 37% survival time of less than 1 min (Fig. 1). This is in accord with previous results (7). The activity of a major internal viral protein, p30, as measured by the micro complement fixation assay (9) after Triton X-100 treatment, shows a relatively great resistance to UV inactivation. After a lag of 90 min, by which time all infectivity has been lost, slow inactivation of p30 activity was detected.

After irradiation endogenous and exogenous viral polymerase activity was assayed as previously described (4, 10). The UV inactivation rates for both activities indicate single-hit kinetics with 37% surviving activity reached by 10 to 13 min (Fig. 2). The inactivation rates of both activities were similar; thus, unirradiated nucleic acid template and primer added in the exogenous reaction did not reverse inactivation.

The polymerase in Triton X-100-treated Rauscher leukemia virus converts 12 to 13 pmol of thymidine triphosphate per h per μg of protein at 37 C to acid-precipitable material. After purification of the enzyme by using the oligo(dT) cellulose column technique of Gerwin and Milstien (2), the activity was increased to 200 to 250 pmol of thymidine triphosphate per h per μg of protein. Triton X-100, which absorbs UV light, was removed by Sephadex G-50 chromatography. The polymerase was then subjected to UV irradiation with a high-intensity water prism monochromator with a Philips SP 500 W high-pressure mercury arc lamp (1) at 254 and 280 nm. If structural damage to the protein were the cause of inactivation, then loss of activity should be greater at 280 nm (6). The rate of loss of activity at 254 nm was about 65% the rate of loss at 280 nm (Fig. 3).

The UV dose at 254 nm resulting in 37% survival of infectivity, as calculated from Fig. 1, is 2×10^3 ergs/mm^2. The dose resulting in 37% survival of viral polymerase activity at 254 nm (Fig. 2) is 2.4×10^4 to 3.1×10^4 ergs/mm^2. This D_{37} is similar to that of carboxypeptidase A (11). Since a viable polymerase is essential for oncornavirus infectivity, at least 5 to 10% of the loss of infectivity of irradiated preparations may be due to viral polymerase inactivation.

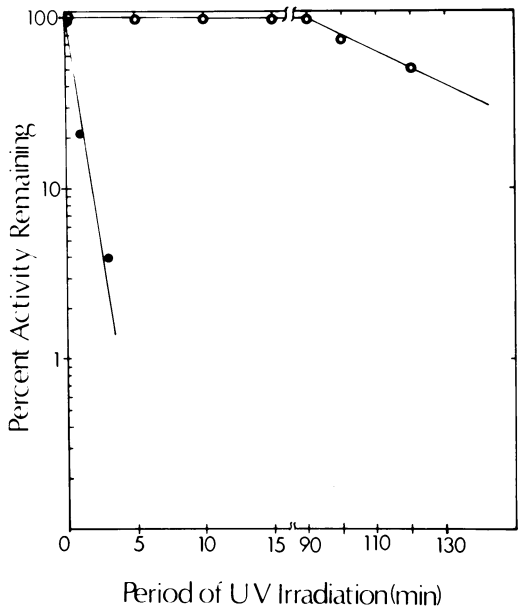

FIG. 1. *Effect of irradiation on infectivity* (●) *and p30 serological activity* (○) *of Rauscher leukemia virus. A suspension of Rauscher leukemia virus was irradiated at 40 ergs per mm² per s, and samples were assayed for infectivity and p30 serological activity as described.*

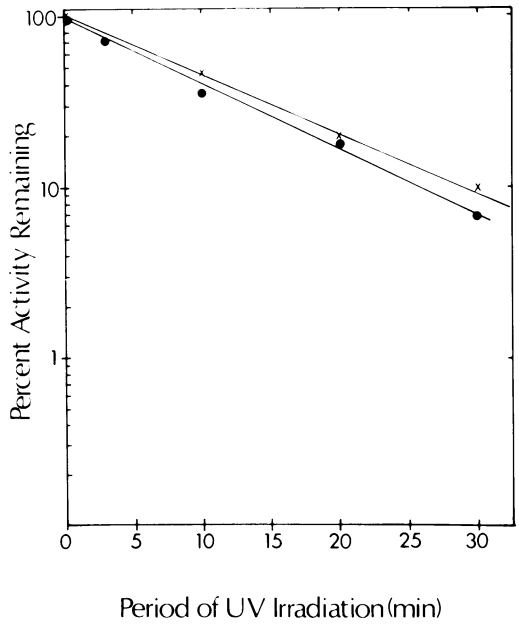

FIG. 2. *Effect of UV irradiation on the endogenous* (×) *and exogenous* (○) *reaction of the viral RNA-directed DNA polymerase. A suspension of Rauscher leukemia virus was irradiated as in Fig. 1. Samples were assayed by the method of Hatanaka et al. (4, 10).*

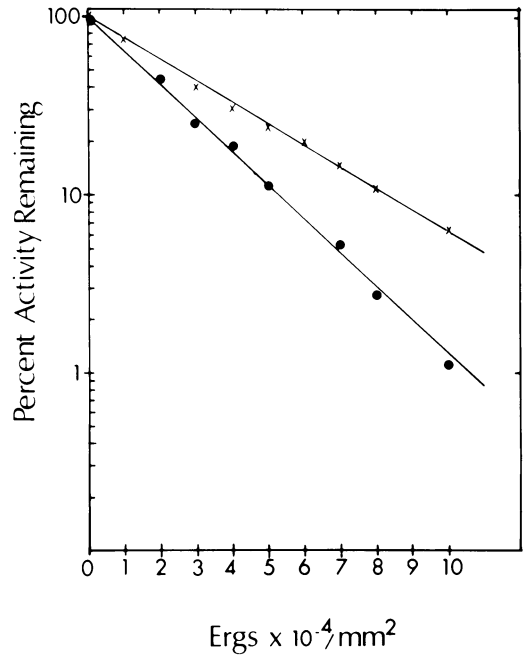

FIG. 3. *Effect of irradiation at 254 nm* (×) *and 280 nm* (●) *on purified viral polymerase. Purified polymerase was freed of Triton X-100 (transmittance at 254 nm = 87.5% transmittance at 280 nm = 83.6). Full (100%) activity was 3,674 counts/min in the exogenous reaction performed by the method of Hatanaka et al. (4, 10).*

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Full (100%) activities (zero times) in the endogenous and exogenous reactions were 773 and 88,237 counts/min, respectively.

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