THE EFFECT OF UV-IRRADIATION ON THE PLAQUE-FORMING ABILITY OF SINGLE- AND DOUBLE-STRANDED POLYOMA VIRUS DNA

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It has been demonstrated by Sinsheimer et al. (1962) that the double-stranded replicative form (RF) of bacteriophage ØX 174 is much more resistant against UV-irradiation than the single-stranded viral DNA with respect to plaque-forming ability. Evidence has been provided by Jansz et al. (1963) and by Yarus and Sinsheimer (1964) that this difference in sensitivity can be ascribed to repair enzymes in the host cell which are effective only on double-stranded but not on single-stranded DNA.

In view of the physical similarity between polyoma and RF DNA, a similar study was undertaken using single- and double-stranded DNA of polyoma virus. This was possible since not only native viral DNA but also artificially prepared single-stranded DNA is infectious for mouse embryo cells, as was first demonstrated by Dulbecco and Vogt (1963) and confirmed in this study. It was found that single-stranded polyoma virus DNA is more sensitive to UV-irradiation than the double-stranded viral DNA.

Materials and methods

Growth of virus and preparation of viral DNA. Polyoma virus strain IL-11 was obtained from Dr. Winocour and grown on mouse kidney cells as described by Winocour (1963). The crude viral

preparation in 0.01 M Tris buffer pH 8.0 was incubated with DNase and RNase, extracted two times with genetron to remove non-viral protein and then banded in CsCl, $\rho = 1.34$. The main visible band at $\rho = 1.34$ was collected and the viral DNA isolated as described by Weil (1961). Phenol was removed by dialysis in SSC (0.15 M sodium chloride - 0.015 M sodium citrate pH 7.3). Usually the DNA preparations contained only one component with a sedimentation coefficient of 20-21 S.

Conversion of 20S to 16S DNA. The 20S component of polyoma DNA was converted to the 16S component using low concentrations of pancreatic DNase. The method was essentially the same as that described by Vinograd et al. (1965). To a sample of 20S DNA (35 μg/ml) in 0.02 M NaCl, 0.02 M Tris pH 8.0, 1/100 vol of a 0.2 M $MgCl_2$ solution and 1/10 vol of a DNase solution containing 0.001 μg/ml were added. The DNase (NBC, 2 x cryst.) was diluted in 0.02 M NaCl, 0.01 M Tris pH 8.0 containing 0.1% bovine plasma albumine (Armour Pharm.Comp.). The sample was incubated at 20°C and the reaction stopped by the addition of 1/10 vol 0.5 M glycine buffer pH 9.8 or by the addition of 1/10 vol 0.1 M sodium citrate. The degree of conversion of 20S to 16S DNA was checked by band centrifugation in the analytical ultracentrifuge. Usually 20-25 minutes of incubation are sufficient to convert 50-60% of 20S DNA to 16S DNA. Separation of the two components of polyoma DNA was achieved by zone sedimentation through a 5-20% neutral sucrose gradient. 0.8 ml DNA solution was layered on top of the gradient in a Spinco SW25 tube and the sample was centrifuged during 16 hours at 24,000 r.p.m. (4°C). Fractions of 0.7 ml were collected from the bottom of the tube.

Single-stranded polyoma DNA was prepared by heat- or alkali-denaturation. 16S DNA in 0.02 M NaCl, 0.05 M glycine buffer

pH 9.8 was heated for 5' at 95° and cooled rapidly in ice, or NaOH was added to a final concentration of 0.1 N and the sample was neutralized after 5' at room temperature. The sample now contains single-stranded rings and rods, as can be demonstrated by band centrifugation in alkaline CsCl ρ = 1.35, pH = 12.4 (Vinograd et al., 1965).

UV-irradiation. Prior to UV-irradiation the DNA samples were dialyzed against 0.01 M sodium phosphate buffer pH 7.2, 0.001 M sodium citrate. 0.1 - 0.2 ml volumes of DNA were irradiated in sterile watch glasses, using a Philips TUV germicidal lamp at a distance of 41 cm. At this distance the sample will receive about 2400 ergs/mm^2 in 100 seconds. Assay of DNA infectivity. Infectivity of polyoma DNA was measured as described by Weil (1961). Primary mouse embryo cells were used when almost confluent or when confluent for not more than half a day.

Results and discussion.

Polyoma DNA was converted for about 90% to the 16 S component and was then fractionated on a sucrose gradient. The slowly sedimenting 16 S peak was isolated, concentrated and dialyzed against 0.02 M NaCl, 0.05 M glycine buffer pH 9.8. The sample was then heat denatured and irradiated as described under methods. Untreated native DNA was also irradiated and the infectivity of both preparations measured. The results indicate that the single-stranded DNA is infectious and that it is more sensitive to UV-irradiation than double-stranded native DNA, as is shown in fig. 1. The lower part of the curve follows inactivation kinetics of double-stranded DNA. This is probably due to contamination with 20 S DNA from the sucrose gradient and/or to renaturation. The

experiments have been repeated several times, yielding essentially the same results. No difference was observed between heat- and alkali-denatured DNA.

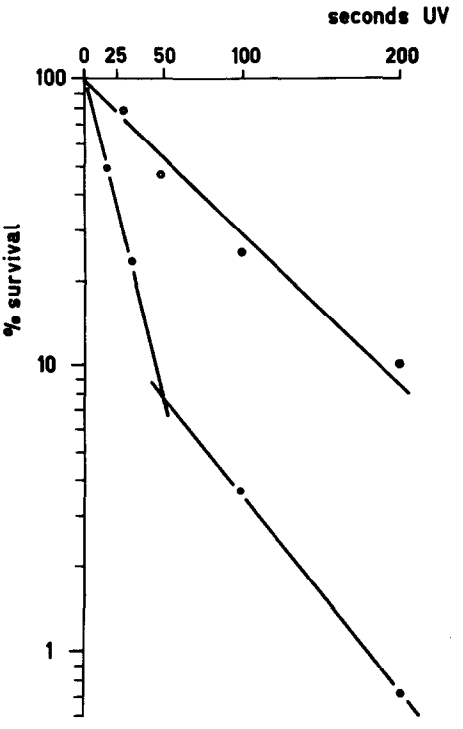

Fig. 1. Inactivation of the plaque-forming ability of single-stranded (•) and double-stranded (o) polyoma virus DNA by ultraviolet light.

At this stage the difference in sensitivity between single- and double-stranded polyoma virus DNA is difficult to interpret. The similarity between the UV sensitivities of single- and double-stranded polyoma DNA on the one hand and of single- and double-stranded ØX DNA on the other hand, suggest that a process similar to host-cell reactivation may operate in polyoma virus infected mouse cells. However, it should be pointed out that thus far all attempts to demonstrate dark repair in mammalian cells of the type observed in microorganisms, have failed (cf. Trosko and Kasschau, 1967). Other explanations of the data reported, such as a difference in intrinsic

sensitivity between single- and double-stranded DNA or an as yet unknown type of repair mechanism, cannot be ruled out.

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