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UV-IRRADIATED SV40 MINICHROMOSOMES AS SUBSTRATES FOR DNA REPAIR ENDONUCLEASES

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UV-irradiated SV40 minichromosomes have been shown to be a substrate for a purified DNA repair endonuclease. A UV-repair endonuclease activity was also found to be associated with the isolated SV40 minichromosomes themselves. It appeared to have similar properties to the enzymes described from other mammalian sources.

Mammalian DNA repair endonuclEases recognizing damage in UV-irradiated DNAs have been extensively purified and characterized from various sources (1-6). One such enzyme isolated from mouse plasmacytoma cells (MPC-11) is active in the absence of divalent metal ions and causes hydrolysis of UV- and γ-irradiated DNA as well as acid and OsO_4-treated DNA (6). It was recently shown that this enzyme preferentially recognizes damage in supercoiled DNA, while UV-irradiated relaxed or linear DNA are not recognized as substrates by the enzyme (7). The exact mechanism for recognition and cleavage of the phosphodiester bond is not known, but the

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enzyme may possibly recognize particular structural features of the UV-irradiated DNA found only in supercoiled DNA.

In all studies carried out previously highly purified supercoiled DNAs have been employed as substrates for the UV-endonucleases. To further study their requirements, the use of substrates resembling those found in vivo would be advantageous. For this purpose SV40 minichromosomes may be useful. This genome is well characterized, and it has well-defined chromatin structure (8,9). Moreover, SV40 minichromosomes can be purified in large amounts and are not contaminated by host DNA (10).

The present study shows that UV-irradiated SV40 minichromosomes can function as substrates for the MPC-11 DNA repair endonuclease. Furthermore, a DNA repair endonuclease that recognizes UV-lesions in DNA was also found to be associated with the SV40 minichromosomes themselves.
This enzyme has properties similar to other purified DNA repair endonucleases.

MATERIALS AND METHODS

Preparation of minichromosomes and incubation conditions.
Growth of CV-1 cells and infection with SV40, strain Rh 911 (40 p.f. u./cell) were carried out as described in reference (11).

The cells were labelled with 10 μCi[^{3}H]-thymidine (25 Ci/mmole)/dish between 25 and 36 hrs, after infection. A nuclear extract free of cellular DNA, but containing SV40 minichromosomes was prepared according to Su and De Pamphilis (10) with minor modifications (12). The minichromosomes were purified using a 5-30% sucrose gradient, the centrifugation period being 3½ hrs in a SW-40 Beckman rotor at 40.000 rpm. The minichromosomes thus isolated were free of radioactivily labelled replicating DNA. [^{3}H] ØX174 RFI DNA and the DNA repair endonuclease from mouse plasmacytoma cells (MPC-11) were prepared and isolated as described by Nes (6). In the experiments reported here, the enzyme activity that eluted at 0.35 M KCl from the phosphocellulose column was used.

Irradiation of the minichromosomes by UV light was carried out employing a Sylvania G8 TS germicidal lamp. The samples to be irradiated were kept in a 1 mm quartz cuvette and exposed to a final dose of 1.1 kJ/m^2. In the ØX174 RFI DNA this exposure gave approximately 35 thymidine dimers/genome.

The output of the lamp was checked with a Laterjet meter before each experiment.

All incubations were performed essentially as described in reference (7) at 37°C and the reaction time is indicated in the legends to the figures. The standard assay mixture contained: 10 mM Tris-HCl (pH 8.0 at 25°C), 10 mM EDTA, 10 mM 2-mercaptoethanol and 100 mM KCl. The activity of the DNA repair endonuclease on UV-irradiated [^{3}H]-ØX 174 RFI DNA was routinely measured using the nitrocellulose filter technique (6).

Alkaline sucrose gradients.

Linear alkaline 5-20% sucrose gradients, 4.8 ml, containing 0.3 M NaOH, 0.7 M NaCl and 5 mM EDTA were prepared in polyallomer tubes. Each tube had a cushion of 0.15 ml 50% sucrose saturated with CsCl. The centrifugation was carried out in the Beckman SW-50 rotor for 70 min at 50.000 rpm and at 20°C. The fractions were harvested from the bottom of the tube and counted in a dioxane-based scintillation liquid in a Packard 460 CD scintillation counter.

RESULTS AND DISCUSSION

Effect of MPC-11 DNA repair endonuclease on UV-irradiated SV40 minichromosomes.

When unirradiated SV40 minichromosomes were subjected to alkaline sucrose gradient centrifugation a single peak of DNA was seen (Figure 1 A-D), corresponding to form I of SV40 DNA with a sedimentation coefficient of 53 S. UV-irradiated SV40 minichromosomes were treated as above and in this case the amount of the form II had increased to approximately 10%, Figure 1 D.

When the UV-irradiated chromosomes were kept at 37°C for 12 minutes prior to the centrifugation the amount of form II DNA was found to be approximately 20% (Figure 1 E), strongly suggesting that the SV40 minichromosomes themselves contain an endogenous UV-endonuclease since there is virtually no degradiation of the unirradiated chromosomes (Figure 1 B). Finally, when MPC-11 DNA repair endonuclease was added to UV-irradiated SV40 minichromosomes and incubated at 37°C for the same period as above approximately 50% of form I DNA were converted to the form II. A similar result was obtained

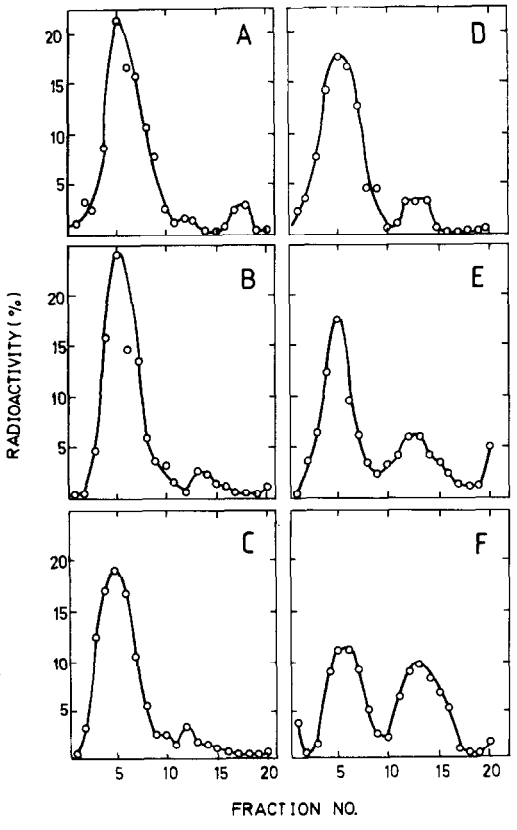

Figure 1. DNA repair endonuclease activity on non-irradiated and irradiated SV40 minichromosomes.
The alkaline sucrose gradients as described in Methods were employed.

A) - C) Non-irradiated minichromosomes
D) - F) Irradiated minichromosomes. 150 µl minichromosomes (1.2 µg DNA, 5400 cpm) were incubated for 12 min at 37°C using standard conditions.
A) and D) Controls not incubated.
B) and E) Incubated without addition of endonuclease.
C) and F) Incubated in the presence of 30 units UV-endonuclease.
The sedimentation is from right to left.

when the UV dose was increased to 2.2 kJ/m^2 (Figure 3). Separate experiments were carried out to study whether the endogenous UV-endonuclease was bound to the SV40 minichromosomes or merely a contaminating protein. The SV40 minichromosomes were therefore recentrifuged in a neutral sucrose gradient (after dialysis) and the UV-endonuclease activity measured in fractions. The major part of the UV-endonuclease

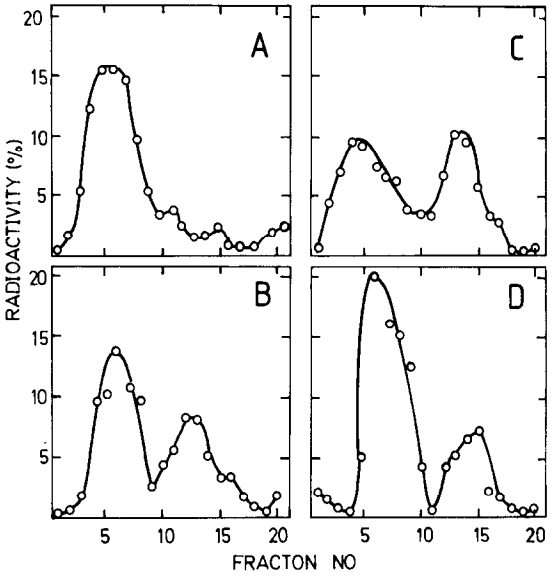

Figure 2. Time course for the degradation of UV-irradiated SV40 minichromosomes. The sucrose gradient analysis were as described in Methods.
The minichromosomes were irradiated with standard dose and incubated at 37°C for different periods.

A) 10 min, B) 20 min, C) 40 min
D) Non-irradiated DNA incubated for 40 minutes.

activity coincided with the SV40 minichromosome peak, strongly suggesting that the activity was bound to the chromosomes. A smaller peak of UV-endonuclease activity was found at the top of the gradient and may represent activity that dissociated off the minichromosomes during the re-centrifugation (results not shown).

The properties of the UV-endonuclease associated with the SV40 minichromosomes were investigated further. Figure 2 shows a rough time course of the reaction. After an incubation period of 40 minutes at 37°C approximately 40% of the DNA in the irradiated SV40 minichromosomes had been converted to form II DNA. When the minichromosomes were exposed to 2.2 kJ/m^2 the convertion of form I to form II increased to 75% (Figure 3) after incubation. With this higher dose,

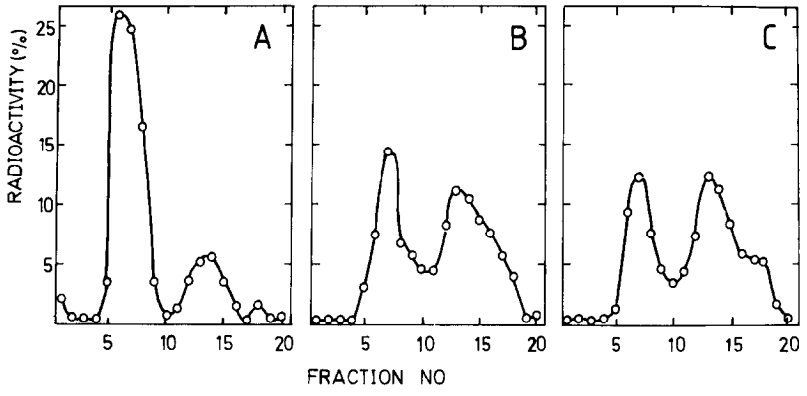

Figure 3. The effect of UV-dose on the stability of SV40 minichromosome DNA.
The alkaline sucrose gradient were as described in Methods. SV40 minichromosomes were irradiated for 30 min (2.2 kJ/m^2). The standard incubation buffer was used.

A) Control not incubated
B) Sample kept for 40 min at 37°C
C) " " " 40 " " " in the presence of 30 units UV-endonuclease.
The sedimentation is from right to left.

however, there was no appearent effect of external enzyme (Figure 3 C). This indicates that the catalysis caused by the minichromosome associated UV-endonuclease is as fast as that caused by the MPC-11 enzyme and that these two enzymes are using the same substrate.

The endogenous UV-endonuclease also attacked UV-irradiated ØX174 RFI DNA as shown in Figure 4. The plateau level obtained was, however, slightly lower than that found for the UV-endonuclease from MPC-11 cells.

The present results show that UV-irradiated SV40 minichromosomes can serve as substrates for the MPC-11 DNA repair endonuclease as well as an endogenous UV-endonuclease. It is at present not clear where the cleavage sites for these enzymes on the minichromosomes are i.e. within the nucleosome or in the linker region. The fact that a higher UV dose did not result in a corresponding increase in form

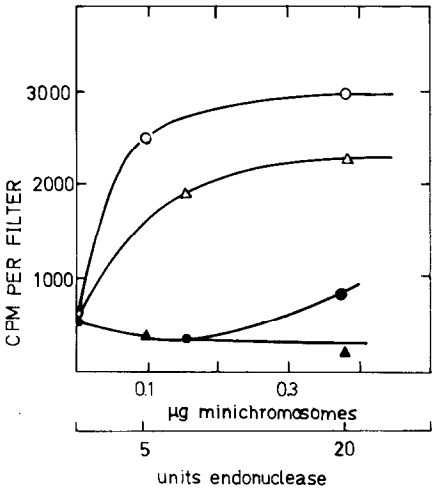

Figure 4. Activity of the SV40 minichromosome-associated UV-endonuclease on ØX174[^{3}H]RFI DNA. 0.2 µg (45000 cpm/µg) non-irradiated (closed) or irradiated (open) DNA in a total volume of 100 µl was incubated in the presence of isolated UV-endonuclease (1 unit/µl) (-Δ-Δ-) or [^{14}C]-labelled SV40 minichromosomes (-□-□-). The mean value of two independent experiments is plotted. The standard nitrocellulose filter assay was used.

II molecules after incubation suggest that the DNA repair endonucleases are limited in their action by certain structural features within the minichromosomes.

The UV-endonuclease associated with the minichromosomes appear to have properties similar to those described for the other mammalian enzymes with regard to none requirement for divalent metalions and high UV doses. Recently, another DNA repair enzyme, uracil DNA glycosylase (12) has also been shown to be bound to the SV40 chromosomes. It is thus possible that in vivo a number of DNA repair enzymes are attached to such chromosomes, which may be useful for studies of DNA repair enzymes in general.

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