ENDONUCLEOLYTIC CLEAVAGE OF UV-IRRADIATED DNA CONTROLLED BY THE V^+ GENE IN PHAGE T4

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SUMMARY

Extracts of E. coli infected with bacteriophage T4 contain a phage-induced UV-specific endonucleolytic activity. This activity is absent from extracts of cells infected with the UV-sensitive mutant T4V1, but is present in another UV-sensitive mutant T4X.

INTRODUCTION

The coliphage T4 has been shown to possess at least two genetic functions that control sensitivity to ultraviolet (UV) light (1). One of these, expressed by the X^+ gene appears to be related both to ultraviolet repair and genetic recombination (2). This function is genetically distinct from a second UV gene designated V^+ (1). Whereas no specific molecular defect has yet been associated with X mutants of T4, a number of workers have demonstrated that the V^+ gene controls an unidentified function(s) associated with thymine dimer excision (3,4). The present studies indicate that the V^+ gene expresses a protein required for endonucleolytic cleavage of UV-irradiated T4 DNA.

MATERIALS AND METHODS

^{3}H-thymine-labelled T4 DNA of specific radioactivity 13,000 cpm/μg was prepared by phenol extraction of phage T4 grown on the host bacterium E. coli B-3 (thy^-). DNA was irradiated with a low mercury vapor pressure UV germicidal lamp at a dose of 1.0×10^5 ergs/mm^2.

Crude extracts of phage-infected cells were prepared as follows: E. coli B-3 was grown to a concentration of 1×10^8 cells/ml in 1.0 liter volumes of nutrient broth containing 0.5% NaCl. Phage was added at a multiplicity of

infection = 3-10. D.L-tryptophan (8 µg/ml) was added to the culture immediately prior to infection. The culture was incubated at 37° for a further 15 minutes at which time chloramphenicol (Parke Davis & Co.) was added to a final concentration of 200 µg/ml. The culture was quickly cooled to 4°C in a dry-ice acetone bath. Cultures were harvested by centrifugation and resuspended in 0.05 M Tris buffer pH 8.0. Extracts were prepared by sonication in a Branson Model W140D sonifier for 45 seconds. Crude extracts were stored frozen. Specific deviations from this procedure are mentioned in the text.

All in vitro studies were done under laboratory illumination by yellow fluorescent lamps (above 5000 Å) to preclude photoreactivation.

The T4 mutants T4V1 and T4X were kindly supplied by Dr. W. Harm.

Deoxyribonuclease activity was measured in crude extracts by two assay procedures:

a. Acid-soluble nucleotide product. Incubation mixtures (1.0 ml) contained 10 µg ^{3}H T4 DNA, (irradiated or native), 1 x 10^{-3} M $MgCl_2$, 100 µg E. coli tRNA, 0.05 M Tris buffer pH 8.0 and 0.1-.47 mg/ml crude extract. Incubations were at 37°C and were terminated by the addition of 0.5 ml 1% bovine serum albumin and 0.5 ml 20% cold trichloroacetic acid. Following centrifugation 1.0 ml of supernatant was added to 10.0 ml of Permafluor (Packard Instrument Co.) in toluene-cellosolve. Radioactivity was measured in a Packard Tricarb Model 3375 liquid scintillation spectrometer.

b. Sedimentation velocity in alkaline sucrose density gradients. Incubation mixtures (0.8 ml) contained 5 µg ^{3}H T4 DNA (irradiated or native), 100 µg E. coli tRNA, 0.05 M Tris buffer pH 8.0 and 0.1-0.22 mg/ml crude extract. Reactions were at 37°C for 10 minutes and were terminated by the addition of 0.1 ml 0.1 M EDTA and 0.1 M 1% sodium lauryl sulfate. A sample (0.05 ml) was layered over a 5-20% alkaline sucrose gradient containing 10^{-3} M EDTA. Sedimentation was carried out in a Spinco type 56 rotor at 20°C. Specific centrifugation conditions are mentioned in the figure legends. Ten-drop fractions were collected directly into individual scin-

tillation vials. To each vial were added 1.0 ml Nuclear Chicago Solubilizer and 10.0 ml of scintillant.

RESULTS

a. UV irradiated DNA. Crude extracts of T4-infected cells degraded UV-irradiated T4 DNA at a significantly greater rate than did uninfected extracts. Evidence for this is an approximately 10-fold increase in the rate of formation of acid-soluble nucleotide (Fig. 1a) as well as the reduced sedimentation rate in alkaline sucrose gradients (Fig. 2a). This increased deoxyribonucleolytic activity is a phage-induced effect requiring active protein synthesis as was demonstrated in the following experiments. Two cultures of E. coli B were infected with phage T4 and protein synthesis was

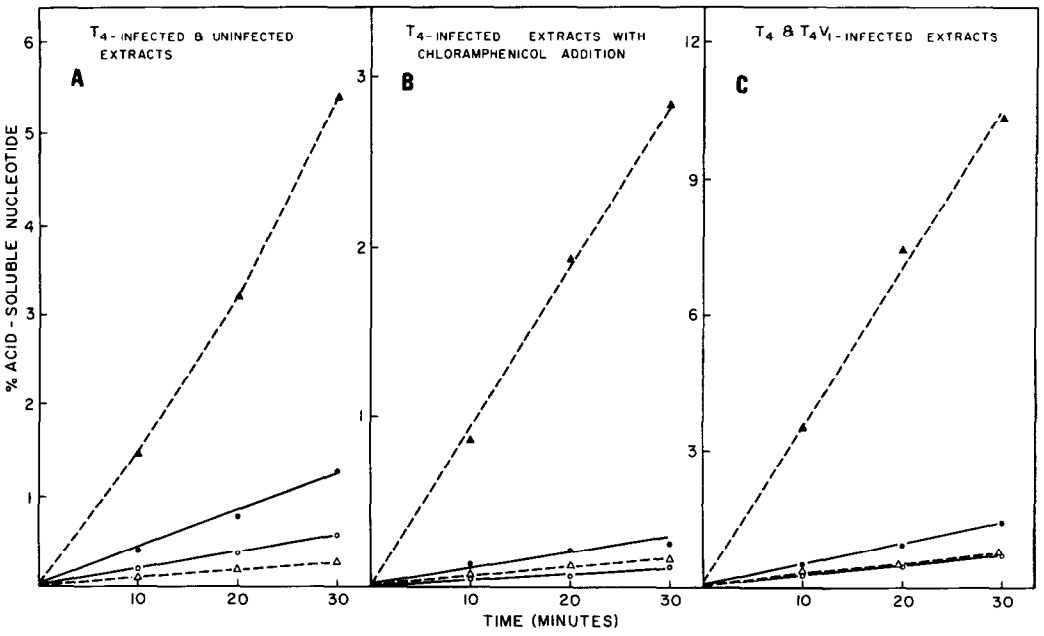

Figure 1. Formation of acid-soluble nucleotide product from UV irradiated and unirradiated ^{3}H-T4 DNA. (See text for further details). Unless otherwise stated, all cultures had chloramphenicol added at 15 minutes after phage addition. In all three figures closed symbols (▲,●) represent irradiated DNA and open symbols (△,○) represent unirradiated DNA. Interrupted lines (- - - -) represent extracts from T4-infected cells.

a) ○,● extracts from uninfected cells.
b) ○,● extracts from T4-infected cells with chloramphenicol addition at time of infection.
c) ○,● extracts from T4V1-infected cells.

inhibited in one culture by the addition of chloramphenicol at the time of infection. The results (Figs. 1b and 2b) show that the crude extract from the control infected culture degraded UV-irradiated DNA more extensively than did the extract from the culture in which protein synthesis was inhibited.

Crude extracts were also prepared from cells infected with the UV-sensitive mutants T4V1 and T4X, and the deoxyribonucleolytic activity of these extracts was compared with that from wild-type T4-infected cells. The extracts from T4V1-infected cells did not degrade UV-irradiated DNA at a rate comparable to the extracts from wild-type infected cells (Figs. 1c and 2c). In fact the extracts from T4V1-infected cells were strictly comparable to uninfected extracts or extracts from infected cells in which protein synthesis was inhibited. On the other hand extracts from T4X-infected cells were indistinguishable from those of wild-type infected cells.

b. Unirradiated DNA. The deoxyribonucleolytic activity present in extracts of T4-infected cells is specific for UV-irradiated T4 DNA and does not degrade unirradiated T4 DNA. This was shown by incubation of crude extracts of infected and uninfected cells with unirradiated T4 DNA. All the crude extracts discussed in the previous section were tested and in every case there was no significant difference in either the rate of formation of acid-soluble products (Figs. 1a,b,c) or in the sedimentation velocity of T4 DNA (Figs. 2a,b,c).

Finally, it should be noted that UV-irradiated T4 DNA was degraded slightly more rapidly than unirradiated DNA by extracts of uninfected cells (Fig. 1a). Preliminary studies indicate that this difference does not result from UV-specific enzymes.

DISCUSSION

The present *in vitro* experiments have shown that crude extracts of T4-infected cells contain deoxyribonuclease activity that is specific for UV-irradiated T4 DNA. Results of the sedimentation velocity studies show that

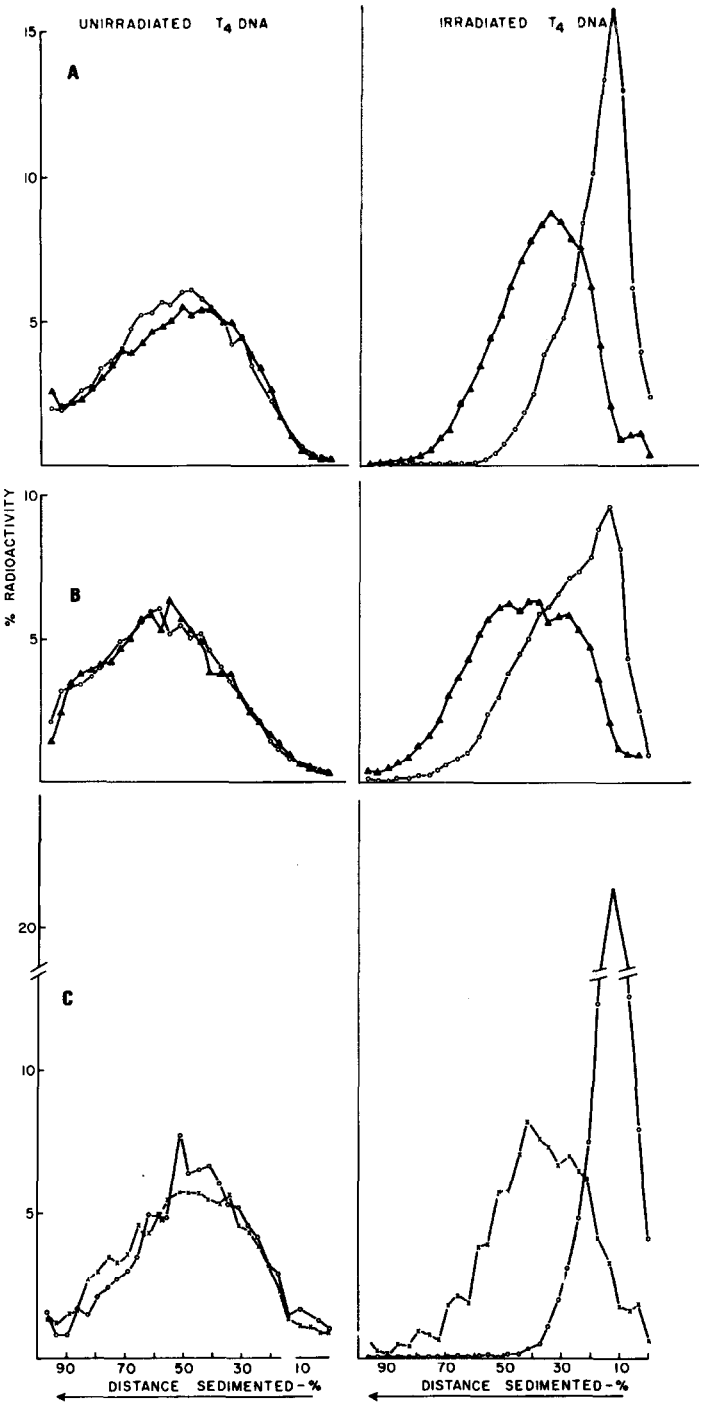

Figure 2. Sedimentation of unirradiated and irradiated ^{3}H-T4 DNA in alkaline sucrose density gradients, following incubation with crude extracts (see text for details). Unless otherwise stated all infected and uninfected cultures had chloramphenicol added at 15 minutes after phage addition.

a) Crude extracts from uninfected cells ▲—▲; T4-infected cells ○—○. Centrifugation at 37,000 rpm for 180 minutes.

b) Crude extracts from T4-infected cells with chloramphenicol addition at time of infection ▲—▲; T4-infected cells ○—○. Centrifugation at 37,000 rpm for 180 minutes.

c) Crude extracts from T4V1-infected cells ✕—✕; T4-infected cells ○—○. Sedimentation at 35,000 rpm for 180 minutes.

this activity is endonucleolytic in nature. If the observed reduction of sedimentation velocity were due to exonucleolytic degradation alone, one would expect the formation of considerably more than the 1% acid-soluble nucleotide product that was observed. The other assay procedure used, viz., the measurement of acid-soluble nucleotide product, is less specific for endonucleolytic activity. However, the complete uniformity in the results obtained using two different procedures indicates that they measured the same activity.

Previous studies on UV-sensitive mutants of phage T4 have demonstrated that the mutant T4V1 does not excise thymine dimers (3,4). The present *in vitro* studies show that the specific defect in phage T4V1 is the absence of an endonucleolytic activity specific for UV-irradiated T4 DNA. This activity is present in extracts of cells infected with either the wild-type phage or the mutant T4X. The *in vitro* demonstration of a phage-induced endonucleolytic function expressed by the V^+ gene is not consistent with the proposed ideas that the genetic defect in phage T4V1 results in the resistance of its DNA to enzymatic repair by the host. These experiments also support the argument that the basis of the UV-sensitivity of the T4X mutant is unrelated to the process of dark repair (2).

The V^+ gene product is presently being purified and characterized. Preliminary studies indicate that the T4 UV endonuclease is also capable of attacking UV-irradiated *E. coli* DNA and is thus not specific for glucosylated DNA.

REFERENCES

1. Harm, W. (1963) Virology, 13, 66.
2. Harm, W. (1966) Mut. Res. 1, 344.
3. Setlow, R. B. and Carrier, W. L. (1966) Biophys. J. 6, 162.
4. Takagi, Y., Sekiguchi, M., Okubo, S., Nakayama, N., Shimada, K., Yasuda, S., Nishimoto, T. and Yoshihara, H. (1968) Cold Spring Harb. Symp. Quant. Biol. 33, 219.
5. Kozinski, A. W. and Lorkiweicz, Z. K. (1967) P.N.A.S. 58, 2109.