# A UV-SPECIFIC ENDONUCLEOLYTIC ACTIVITY PRESENT IN HUMAN CELL EXTRACTS*

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Received June 29,1972

Summary: An endonucleolytic activity specific for UV-irradiated DNA has been detected in extracts of human cells, both normal and defective in the repair of UV-damage. The activity is apparently recognizing a UV-induced damage other than pyrimidine dimers.

In view of the key role played by DNA in the life and reproduction of organisms, it is not surprising that mechanisms to insure its integrity have evolved. The repair of UV-induced damage has been demonstrated in a variety of organisms of both microbial and mammalian origin (see 1 and 2 for reviews). One of the known mechanisms by which this repair occurs in bacteria is "excision repair" which has been studied mainly in connection with the removal of pyrimidine dimers (3). This model of repair has been extrapolated to mammalian systems where excision of dimers in vivo has been observed (4). By analogy with the UV-sensitive bacterial mutants, it has been assumed that the cells from Xeroderma pigmentosum and De Sanctis Cacchione patients (which are more UV-sensitive and less able to repair UV-damage than normal cells) are defective in the enzyme(s) involved in

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* Work supported by Euratom (grant no. 052-65-2 BIAN)

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Abbreviations: XP, Xeroderma pigmentosum; Ad 5, Adenovirus 5; UV-DNA, UV-irradiated DNA; Mw, molecular weight; PR, photoreactivating enzyme.

the recognition and/or removal of the dimers (4).

Repair mechanisms other than excision repair (e.g. recombination repair) could also be present in mammalian cells and possibly be even more efficient than the excision repair pathway. Furthermore, different repair mechanisms could be operating on different types of UV-damage.

Some of the enzymes which are thought to play a role in the excision repair in bacteria have been isolated and purified (see 5) but none so far has been identified in mammalian cells. The present report deals with an endonucleolytic activity specific for UV-irradiated DNA which we have detected in human cell extracts in the course of our studied on mammalian DNA repair enzymes.

Methods.-- HeLa S3 as well as SV40-transformed primary fibroblasts from a Xeroderma patient (XP9) and his phenotypically normal heterozygote mother (XP10) were grown in monolayers in Eagle's MEM (with Earle's salts) supplemented with 10% calf serum or fetal calf serum. Untransformed primary fibroblasts from a De Sanctis Cacchione patient (XP25) were grown in F12 medium with 15% fetal calf serum. The heterozygote XP10 cells have a normal UV-repair activity as determined by unscheduled DNA synthesis, while the XP9 cells perform about 10% of the unscheduled DNA synthesis of control cells (6). The XP25 cells have an undetectable level of unscheduled DNA synthesis (de Weerd-Kastelein, unpublished data).

Crude extracts were prepared by sonicating the cells (2-5 x $10^8$ cells/ml in 10 mM Tris-1 mM EDTA, pH 7.5) with two 40 sec pulses (at a peak amplitude of 8 microns in a MSE sonicator) and removing the cell debris by centrifugation (15 min; 105,000 x g). $^3H$- and $^{14}C$-labelled Adenovirus 5 DNA was isolated from Adeno-

virus 5-infected BSC-1 cells. This DNA had a single stranded molecular weight of $1.15 \times 10^7$ dalton as determined by alkaline band sedimentation in the Spinco Model E analytical centrifuge. The DNA was irradiated in Tris-EDTA under a 30 w Philips germicidal lamp (maximum intensity at 254 nm) with a total exposure of 5000 ergs/$mm^2$, unless otherwise indicated. The assay mixture contained: 85 µl of 10 mM Tris- 1 mM EDTA, pH 7.5; 15 µl of E. coli sRNA (6 mg/ml in 10 mM $KH_2PO_4$- 1 mM EDTA, pH 7.5); 50 µl (0.25 µg) of Ad 5 UV-$^3$H-DNA and 50 µl (0.25 µg) of Ad 5 unirradiated $^{14}$C-DNA as internal control. In some experiments $^{32}$P-labelled RF DNA from phage ΦX174 was used as substrate instead of Ad 5 DNA. The reaction was started by addition of the extracts (50 µl containing 800-1000 µg cellular protein as measured by the absorbance at 260 and 280 nm (7) ). Blanks without extract or with boiled extract were incubated as controls. After incubation at 37°C for 60 min, the products of the reaction were analyzed by centrifugation on alkaline sucrose gradients (5-23%, pH 12.8). The molecular weight of the UV-DNA was calculated from the mode of the sedimentation profiles assuming a Mw of $1.15 \times 10^7$ dalton for the unirradiated $^{14}$C-DNA and that the distance sedimented is proportional to the Mw raised to the power 0.4 (8).

Photoreactivation of the UV-DNA was carried out in 0.15 M NaCl-0.015 M Na-citrate, pH 7.0 for 30 min at 37°C under 2 Philips TL 20w/08 lamps at a distance of 20 cm.

Results and Discussion.-- In the first experiment, $^3$H-labelled Ad 5 DNA was irradiated with UV light and incubated with extracts from HeLa or XP cells. From the data presented in Fig. 1 it is clear that after incubation with the cell extracts the sedimentation of the unirradiated $^{14}$C-DNA is not altered while the UV-

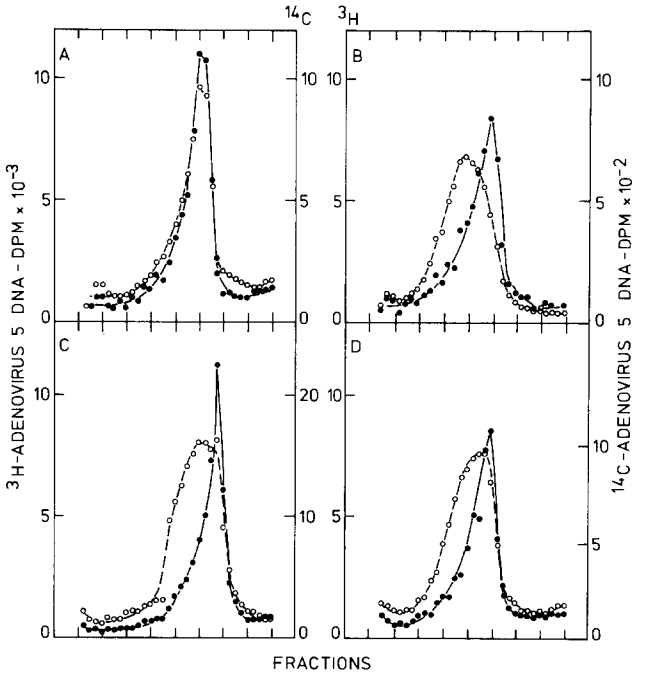


Fig. 1. Sedimentation profiles of Ad 5 $^{3}H$-DNA irradiated with UV light (5000 ergs/mm$^2$) and incubated with extracts from human cells. Panel A: no extract added; Panel B: HeLa cell extract; Panel C: XP9; Panel D: XP25.

●———● unirradiated $^{14}C$-DNA
o---------o UV-$^{3}H$-DNA

Centrifugation was for 7 hrs at 41 000 rpm in a SW41 rotor of a Spinco L2 centrifuge. The direction of sedimentation is from left to right.

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$^{3}H$-DNA sediments at a slower rate than the unirradiated DNA. Irradiation alone (panel A) does not affect the sedimentation properties of the $^{3}H$-DNA. The reduction in molecular weight for the UV-DNA is not significantly different for the four cell lines studied (HeLa: 7.6 x $10^6$ dalton; XP9: 8.6 x $10^6$; XP25: 8.9 x $10^6$; XP10 (not shown): 8.5 x $10^6$). These data, therefore, show the presence of an activity in extracts from human cells (both normal and defective in UV-repair) which acts on UV-DNA but does not significantly affect unirradiated DNA. Similar activi-

ties were obtained from cells in logarithmic or stationary phase of growth and from frozen cells; attempts to stimulate the activity by UV-irradiation of the cells prior to preparation of the extract were unsuccessful. The activity was found stable for at least a week upon storage of the extracts at $0^{o}C$ or $-20^{o}C$. Boiling of the extracts for 5 min resulted in loss of all activity.

That the activity may be of endonucleolytic nature can be inferred from the observation that no material of low molecular weight (such as would be produced by an exonuclease reducing the Mw to the extent observed) is found at the top of the gradients. Instead all of the input radioactivity is recovered under a peak which is broader and partly displaced towards the top of the gradients. A further confirmation of the endonucleolytic nature of the activity is provided by the data on the conversion of ΦX174 RFI DNA into RFII DNA (Table I). Irradiation alone has no effect on the relative percentages of RFI and RFII forms. Incubation with the extract does not introduce single strand breaks into the unirradiated DNA but clearly affects the UV-DNA. The activity is lost upon boiling of the extract prior to incubation with the DNA. The magnitude of the effects shown in Fig. 1 and Table I increases with extract concentration, length of incubation at $37^{o}C$ (at least up to 120 min) and UV-exposures (between 1000 and 10000 ergs/$mm^2$).

In order to investigate the site of action of the activity in question, the UV-DNA from Ad 5 was incubated with a purified preparation of photoreactivating enzyme (PR) from Streptomyces griseus prior to incubation with the cell extracts. If the mammalian cell extract acts at the dimers, the photoreactivated UV-DNA would not be a specific substrate since the PR enzyme monomerizes the thymine dimers. As a control for the action of

Table I

Conversion of ϕX174 RFI into RFII after incubation with an extract from HeLa cells

| | % RFI | % RFII |
|---|---|---|
| Unirradiated RF-no extract | 35 | 65 |
| UV-RF ( 5000 ergs/mm$^2$)-no extract | 36 | 64 |
| UV-RF (10000 ergs/mm$^2$)-no extract | 32 | 68 |
| Unirradiated RF-HeLa extract | 35 | 65 |
| UV-RF ( 5000 ergs/mm$^2$)-HeLa extract | 23 | 77 |
| UV-RF (10000 ergs/mm$^2$)-HeLa extract | 14 | 86 |
| UV-RF (10000 ergs/mm$^2$)-HeLa boiled extract | 35 | 65 |

The relative percentages of RFI (double stranded closed circles) and RFII (single stranded circles and linear forms) were determined by alkaline sucrose velocity sedimentation (1 hr 45 min at 60 000 rpm in a SB405 rotor of an International B60 centrifuge)

the PR enzyme, the UV-specific endonuclease purified from Micrococcus luteus which specifically recognizes the dimers has been used. From the data in panels A-B of Fig. 2 it is clear that after photoreactivation the breakdown of the UV-DNA by the endonuclease from M.luteus is greatly reduced. Although under the present conditions no complete photoreactivation was obtained, the single stranded Mw of the photoreactivated DNA (panel B: $5.3 \times 10^6$ dalton) is 7 times higher than the Mw of the non-photoreactivated UV-DNA (panel A: $7.7 \times 10^5$ dalton). However, when one considers the data of panels C-D, no significant effect of photoreactivation upon the activity of the extract from XP10 cells is visible. Similar results were obtained with the other cell lines.

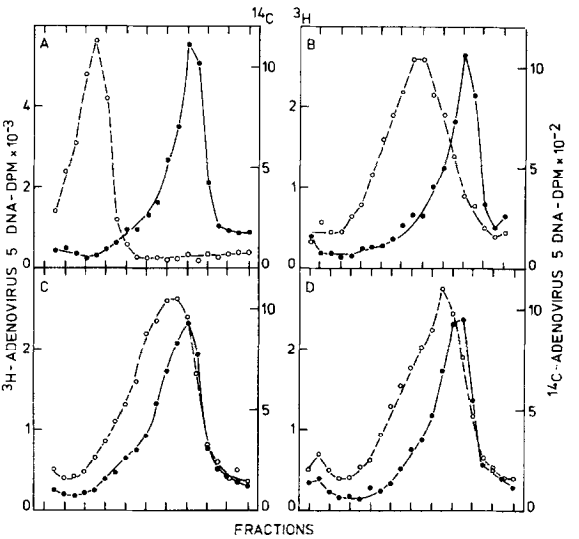


Fig. 2. Sedimentation profiles of Ad 5 UV-$^{3}$H-DNA (5000 ergs/mm$^{2}$) treated with:

Panel A: UV-endonuclease from M.luteus; Panel B: PR enzyme and UV-endonuclease from M.luteus; Panel C: extract from XP10 cells; Panel D: PR enzyme and extract from XP10 cells.

●———● unirradiated $^{14}$C-DNA
o----------o UV-$^{3}$H-DNA

Centrifugation was for 2 hrs at 60 000 rpm in a SB 405 rotor of an International B60 centrifuge. The direction of sedimentation is from left to right.

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The conclusion suggested by these data is that the endonucleolytic activity detected in human cells is not acting at the dimers but may nevertheless be involved in the repair of some other UV-damage. Xeroderma cells, therefore, could still be defective in the enzyme which recognizes dimers but may be able to recognize and possibly repair other forms of UV-damage as efficiently as normal cells. Regarding the possible site of action of the activity reported in this paper, any of the several photoproducts induced by UV-light could serve as substrates and further work is in progress to determine the type of damage specifically recognized under the experimental conditions described.

Acknowledgements.-- We wish to thank Dr. F.L. Graham (Laboratory for Physiological Chemistry, State University of Leiden) for the Adenovirus 5 DNA; Mr. H.L. Heijneker (Medical Biological Laboratory TNO, Rijswijk) for the ΦX174 RF DNA; Dr. R.A. Oosterbaan (Medical Biological Laboratory TNO, Rijswijk) for the UV-endonuclease from M.luteus; and Drs. A. Eker and A.M. Fichtinger (Institute of Biochemistry and Biophysics, University of Delft) for the PR enzyme. We are also grateful to Drs. P.H. Pouwels and R.A. Oosterbaan for helpful suggestions and revision of the manuscript.

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