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Note

The *in vivo* Effect of Ultraviolet Irradiation (290—350 nm) on Epidermal Chromatin

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In vivo ultraviolet radiation (290—350 nm, 800 J/m²) in the absence of any exogenously added sensitizer induced cross-linking of DNA to protein in chromatin isolated from guinea pig epidermis. The chemical properties and the biologic activity of epidermal chromatin isolated from both irradiated and non-irradiated specimens were examined. One hour post-irradiation the lesion was no more detectable, indicating that a repair process was effective in the viable mammalian epidermis.

Ultraviolet (uv) radiation of wavelengths shorter than 350 nm from sunlight and artificial sources is known to have profound effects on a number of cellular functions. Deoxyribonucleic acid is the most sensitive target of uv photons[1]. The relative yields of different types of photo-products that are produced in DNA, however, can vary depending upon the type of organism used, the irradiation conditions, wavelengths, and the physical state and the environment of the DNA during irradiation. Although the thymine dimer is still the most thoroughly studied lesion shown to be of great biological importance, there is an ever increasing number of observations being reported which suggest that there are other types of uv damage responsible for biological alterations[2]. As DNA does not exist in a cell in pure solution, but is in intimate contact with proteins, RNA, lipids, and other biomolecules, it is conceivable that photochemical heteroadducts can occur and play a role in the radiation induced molecular and pathological lesions to cells.

The cross-linking of DNA and protein in bacteria was the first *in vivo* photochemical heteroadduct reaction to be reported[3]. There have been some studies reported on uv induced DNA and protein cross-links in mammalian cells which were mainly based on the reduced DNA extractability after uv radiation[4,5]. Evidence that this lesion plays a significant role in the killing of uv irradiated cells has been shown for several experimental conditions[2].

The chemical nature of the DNA-protein cross-links is as yet not known. An *in vitro* photochemical reaction between thymine and cysteine has been observed which may be one of the possible mechanisms for the covalent linking of DNA to protein *in vivo*[6]. Furthermore, 11 of the common amino acids have been shown to combine photochemically with uracil in different model systems[7].

All these findings suggest that uv irradiation can induce significant alterations of the chromosomal material, the chromatin. Bearing this in mind, we focused our investigations in the present work on the isolation of chromatin from irradiated and non-irradiated epidermal cells of guinea pig skin. For this purpose the backs of 6 adult albino guinea pigs (700—900 g) were epilated[8], and 6 days post-epilation, irradiated under a 400 watt high pressure mercury lamp equipped with a replaceable yellow cellophane filter which trasmitted wavelengths 290—350 nm. The back of each animal was divided into two equal sections prior to irradiation. The right section (3.5 × 10 cm) was irradiated, and the left half served as a non-irradiated control. Each animal received a dose of 800 J/m² which was equivalent to approximately 5 times the minimal erythema dose (MED)*. Animals were sacrificed immediately after irradiation, and 60 minutes after irradiation. The epidermis, including the horny layer, of irradiated and non-irradiated skin was isolated by the stretch method[8], and homogenized. Chromatin from the so obtained epidermal homogenates was isolated according to Bonner's procedure[9], and purified by centrifigation in 2M sucrose solution. The isolated epidermal chromatin showed an optical density ratio (260/230) of 0.95. Its transcription ability was studied with *E. coli* RNA polymerase according to Burgess' method[10], and was found to be 18—23% as compared to free epidermal DNA. Fractionation and dissociation of the chromosomal complex were achieved by column chromatography on sepharose B4-200 and DEAE cellulose columns with 2M NaCl as the eluant. Optical densities at 260 and 230 nm were obtained for each fraction. Changes in molecular weight of DNA isolated from uv irradiated and non-irradiated epidermis were examined by centrifugation of the isolated DNA on a sucrose density gradient (5—25%; SW 29 rotor, 6 hours at 25,000 rpm), and the sedimentation profiles were compared with a standard DNA of known molecular weight, (Thymus DNA, M. W. 7.2×10^6).

Figure 1 shows the fractionation profile of epidermal chromatin on a DEAE cellulose column. Fraction 1 represents protein fraction that contains nicotinamide adenine dinucleotide (NAD). Fraction 2 contains proteins and traces of NAD and RNA. We could also detect similar amounts of NAD after fractionation of human skin. Fraction 3 is the DNA devoid of protein, and fraction 4 represents a nucleo-protein complex. This last fraction could not be dissociated with 2M NaCl, and this finding constitutes an interesting characteristic of epidermal chromatin.

Figure 2 shows a representative fractionation profile of epidermal chromatin on sepharose B4 columns before and after uv irradiation. The major changes upon irradiation are seen in the first peak, which represents epidermal DNA. In the non-irradiated specimen (A), the optical density ratio (260/230) is 1.99, whereas in the lower curve the optical density ratio dropped to about 1. This drop in optical density ratio as well as the presence of proteins (8—11%) as determined by Lowry's test in this fraction, indicate the cross-linking of DNA and protein. Peak 2 represents histones and acidic proteins. Peak 3 is the stable DNA-protein complex. This fraction is apparently not affected by uv irradiation, and is presently under investigation for its physical-chemical properties.

* The MED is defined as the minimal dose of uv irradiation (290—320 nm) that produces definite, but minimally perceptible redness, at 24 hours after exposure.

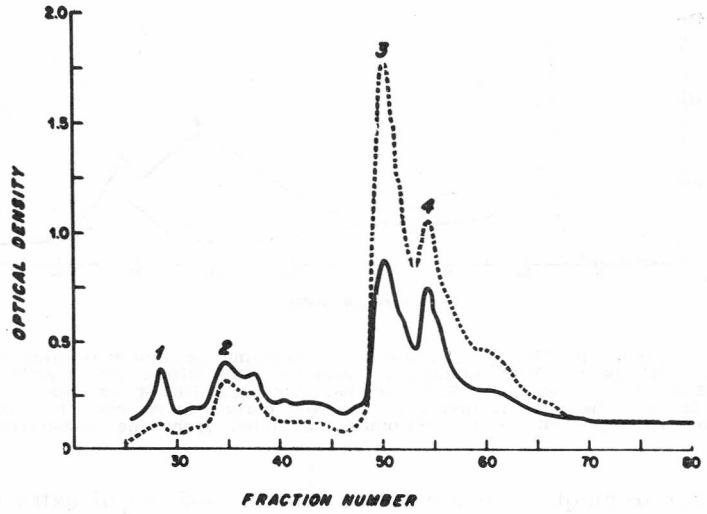

Figure 1. Fractionation profile of skin chromatin on a DEAE cellulose column (1.5 × 120 cm). Elution: linear gradient, 250 ml of 0.001 M sodium phosphate pH 7.2 to 2 M NaCl in sodium phosphate, pH 7.2. Fraction volume 5.0 ml/20 min. OD at 260 nm (— — —) OD at 230 nm (———). Fraction 1 represents protein and nicotinamide adenine dinucleotide (1 mole NAD per 50 moles nucleotides). Fraction 2 is protein, traces of NAD and RNA. NAD was determined on the basis of the characteristic absorption of its reduced derivative at 340 nm. Fraction 3 is DNA; Fraction 4 is the stable DNA-protein complex. Fraction 4 contained 7—9% protein and had a transcription ability of 7—8% as compared to free epidermal DNA.

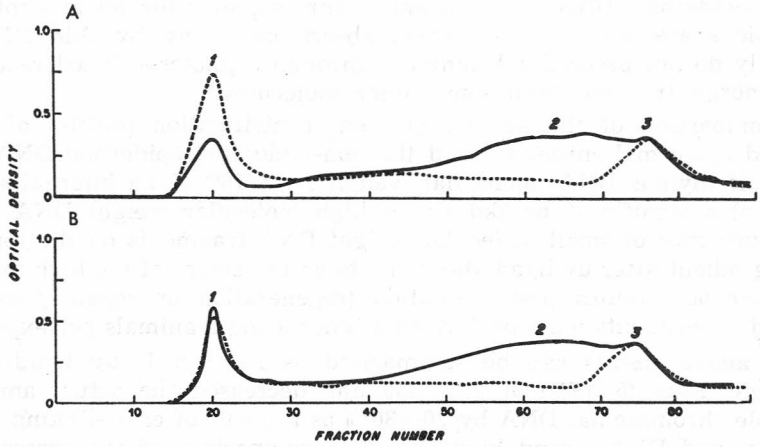

Figure 2. Fractionation profile of epidermal chromatin on a sepharose B4 (2 × 40 cm) before (A) and after (B) uv irradiation. Irradiation dose 800 J/m², 290—350 nm. Eluant 2 M NaCl, in 0.1 M EDTA, fraction volume 2.0 ml, flow rate 1/ml/min. OD ratio 260/230 of peak 1 in A = 1.99, in B 1.0. Number 2, fraction 50—65 represents histones and acidic proteins. Number 3 is the stable DNA-protein complex.

The fractionation profile of chromatin isolated from epidermis one hour after uv irradiation is shown in Figure 3. The optical density ratio of peak 1 is back to 1.99, the original control level, indicating that an active repair process has resulted in the elimination of the DNA-protein cross-linkage. The chromatin pattern in peak 3 did not show any significant changes, again suggesting that this DNA-protein complex is unaffected by uv radiation.

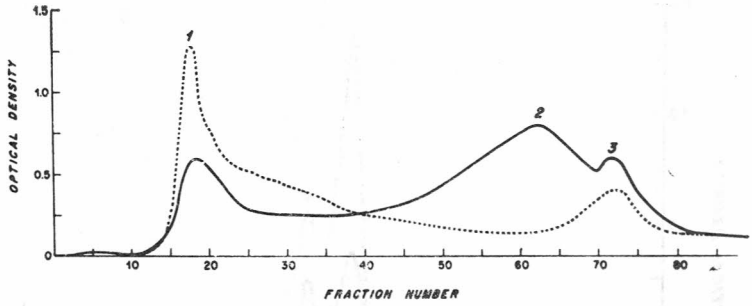

Figure 3. Fractionation profile on sepharose B4 of epidermal chromatin 60 minutes post-irradiation, (regeneration phase). Peak number 1 represents DNA with a 260/230 ratio 1.99; number 2, fraction 52—67, is histones and acidic proteins; number 3, DNA-protein complex. The physical properties and the transcription ability of the chromatin material isolated 60 minutes postirradiation corresponded to the chromatin obtained from the non-irradiated control specimen.

With our techniques we are able to obtain 4—5 mg of extractable DNA free of protein from 1 gram of wet epidermal tissue. Immediately after uv radiation the yield of this extractable DNA is reduced by 20—30%, presumable as a result of DNA to protein cross-linking, and possibly to DNA strand breakage. This later observation is consistent with our previously reported findings[8], as well as with the reported findings of single and double strand breakage of DNA upon uv ($>$ 290 nm) irradiation[16]. We also suggested that the epidermal DNA is the primary chromophore for uv absorption. Its main lesions are the result of direct absorption of uv by skin DNA, and apparently do not proceed substantially through a photosensitized reaction by triplet energy transfer from some other molecules[11].

A comparison of the sucrose gradient centrifigation profiles of the uv irradiated epidermal chromatin and the non-irradiated epidermal DNA, including a calf thymus DNA (molecular weight 1.3×10^6) as an internal standard showed: a) a significant breakdown of high molecular weight DNA fraction and the presence of small molecular weight DNA fragments on the top of the sucrose gradient after uv irradiation; and b) an increment of the high molecular DNA after 60 minutes post-irradiation (regeneration or repair phase). The described experiments were performed 3 times using 2 animals per experiment.

The above results can be summarized as follows: 1) uv irradiation in physiologic doses (5 MED) of 290—350 nm, decreased the actual amount of dissociable chromosomal DNA by 20—30% as a result of cross-linking of DNA to protein and DNA strand breakage; 2) a comparison of the corresponding elution profiles from sepharose columns of the dissociable DNA isolated from uv irradiated and non-irradiated epidermal specimens indicated cross-linking of protein to DNA; 3) uv irradiation caused a significant breakdown of high molecular weight DNA that was isolated upon irradiation, and 4) in the regeneration phase there was an active repair process operating in the viable cells of the epidermis. The mechanism of this repair process is presently under investigation.

The role of NAD in skin is being carefully investigated, as this molecule could serve as a chromophore that transfers energy into an enzyme(s) or other cell constituent for which it is a cofactor, leading to the alteration of

that enzyme. Recently it was shown that reduced nicotinamide adenine dinucleotide (NADH) is highly resistant to uv (300—350 nm) *in vitro*[12].

Hitherto the cross-linking of DNA to proteins was reported only in chemical model systems[6,13], bacterial systems[3], or *in vitro* systems of tissue culture[4,5], and was not examined in a mammalian system *in vivo*. Furthermore, this lesion, as our experiments show, already occurs under physiologic conditions when skin is irradiated with moderate doses of uv light that are normally encountered by man. It is known that diseases and disorders resulting from repeated exposure of human skin to sunlight are manifold[14], and include basal and squamous carcinomas, skin ageing, actinic elastosis, and many photosensitive reactions.

So far, no evidence for the cellular repair of DNA-protein heteroadducts has been found. Han[15], in a recent work on DNA-protein crosslinking in synchronized HeLa cells exposed to uv (254 nm), reports that no recovery from this damage was observed. Our *in vivo* findings, however, suggest that cross-linking of DNA to protein in mammalian skin, induced by wavelengths 290—350 nm is repairable.

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SAŽETAK

Djelovanje ultravioletnog ozračivanja (290—350 nm) na kromatin epiderma *in vivo*

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Ozračenje ultravioletnim svijetlom (290—350 nm; 3×10^5 erg/cm^2) dovelo je do umreženja DNA i proteina u kromatinu izoliranom iz epidermisa zamorca. Jedan sat nakon ozračenja, ta se lezija više nije mogla dokazati, što govori u prilog tomu, da u koži sisavca postoji aktivni mehanizam oporavka, koji prepoznaje i eliminira kovalentnu vezu DNA-protein.

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