DNA IN CHROMATIN OF IRRADIATED LYMPHOID TISSUES DEGRADES IN VIVO INTO REGULAR FRAGMENTS

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1. Introduction

A few hours after irradiation in vivo, the cells in lymphoid tissues undergo serious changes. The pyknotic degeneration of cell nuclei appears, which later leads to so called intermitotic death of the cells. The pyknotic degeneration is preceded and/or accompanied by various signs of chromatin damage. A fraction of chromatin turns soluble in 0.14 M NaCl solution (earlier described as a fraction of released polydeoxyribonucleotides [1,2]. Chromatin could be more easily deproteinized [3], loses a fraction of histones [4] and becomes more sensitive to the action of polyanions [5], deoxycholate [6] and alkaline solution [7]. All these changes show a parallel time course (appearing at 1–2 h, reaching a maximum 6–8 h after irradiation) as well as a parallel dose dependence (almost linear increase after exposure 0–300 R). They occur only in lymphoid tissues and the process leading to these changes develops only under conditions in vivo. The proper mechanism of the development of the post-irradiation damage to chromatin is still unknown; there seems to be no direct relation to the origin and repair of DNA single-strand breaks caused by radiation [8]. Usually, it is assumed that chromatin changes are due to the action of enzymes released after irradiation [9,10].

Recently, the subunit structure of chromatin has been proved in both electron microscopic [11] and biochemical studies. Digestion of chromatin in liver nuclei by endogenous nuclease has been shown to degrade chromatin into regular fragments containing DNA molecules with the concrete size of multiples of a unit weight [12]. Later on micrococcal nuclease was found to digest chromatin from various animal and plant sources to similar subunits [13–15]. It is worth mentioning that regular fragments of nuclear DNA have been found earlier [16] in embryonic mouse liver cells upon 16 h cultivation in vitro.

In the present study we wish to report on experiments demonstrating that chromatin DNA in lymphoid tissues of irradiated mice degrades in vivo into regular fragments. According to the size of DNA, these fragments could not be distinguished from DNA fragments in the subunits released by limited digestion of nuclei by micrococcal nuclease. The results thus confirm the enzymic nature of post-irradiation damage to chromatin. On the other hand, this is the first time that degradation of chromatin DNA into regular fragments has been proved to take place in vivo in tissues of a multicellular organism, though after the action of a strong pathogenic agent.

2. Materials and methods

2.1. *Experimental animals and irradiation*

Non-inbred H strain female mice, aged 6–8 weeks, were used. They were whole-body irradiated by TUR apparatus (V.E.B. Dresden, GDR) at 180 kV, 15 mA, 0.5 mm Cu filtration, 60 R/min. The whole-body exposure was 600 R. Mice were killed by decapitation and the tissues (thymus, spleen, liver) removed for experiments.

2.2. *Isolation of nuclei and of the salt-soluble fraction of chromatin*

Nuclei were isolated by gentle homogenization of tissues in 0.25 M sucrose, 5 mM $CaCl_2$, 5 mM Tris–HCl solution, pH 7.2 and spun down by centrifugation

at 600 × *g* for 7 min. The salt-soluble fraction of chromatin [2] was obtained as supernatant after homogenization of the tissues directly in 0.14 M NaCl solution and centrifugation (2500 × *g*, 15 min).

2.3. *Digestion of nuclei by micrococcal nuclease*

The isolated nuclei from tissues of normal mice were suspended in digestion buffer (0.25 M sucrose, 1 mM $CaCl_2$, 5 mM Tris–HCl, pH 7.4). The suspension of nuclei (containing approximately 2 mg DNA/ml) was then digested with micrococcal nuclease, 7.5 U/ml (Worthington, USA) at 37°C for various intervals (1–10 min). The digestion was stopped by addition of EDTA to the final concentration 2 mM and by chilling on ice.

2.4. *Isolation of DNA fragments and their electrophoresis*

Nuclei from tissues of normal and irradiated mice, as well as nuclei after limited digestion by micrococcal nuclease, were lysed in 0.2 mM EDTA, pH 7.0. Nuclear suspension was frozen and thawed to facilitate the lysis. The supernatant obtained after centrifugation (4000 × *g*, 5 min) as well as the supernatant from tissue homogenate in 0.14 M NaCl (containing salt-soluble chromatin fraction) were deproteinized at 4°C. (Dissociation in 1% SDS and 1 M NaCl solution, stirring overnight, extraction with the same volume of chloroform–isoamylalcohol 24:1.) The DNA from the water phase was precipitated by ethanol overnight at −30°C. DNA was dissolved in electrophoresis buffer containing urea. Electrophoresis was performed according to Loening [17] in 4% polyacrylamide gels. Gels were stained by 0.008% Stains-all (Serva, FRG) in 80% formamide overnight and destained in water. The scanning was performed at 540 nm in SP 1809 densitometer accessory to SP 1700 spectrophotometer (Pye-Unicam, UK).

3. Results and discussion

3.1. *Fragmentation of chromatin in irradiated lymphoid tissues*

Lysis of isolated nuclei from thymuses and spleens of mice killed 1, 2 and 4 h after irradiation caused a release of a fraction of nuclear chromatin from nuclei to the supernatant. The size of DNA molecules obtained after deproteination of this fraction from thymuses, as revealed by polyacrylamide electrophoresis, is shown in fig.1. DNA isolated in this way from tissues of unirradiated, normal mice, remains near the start. DNA isolated from thymus nuclei of irradiated mice shows a regular migration pattern with multiple bands, similar to that described after the action of endogenous [12] or micrococcal nuclease [13–15]. The fragmentation of chromatin DNA into regular segments is apparent as early as 1 h after irradiation; the relative amount of fragments in low classes increases in later post-irradiation intervals (2 h and 4 h). Similar DNA fragments could be isolated also from the salt-soluble fraction of thymus

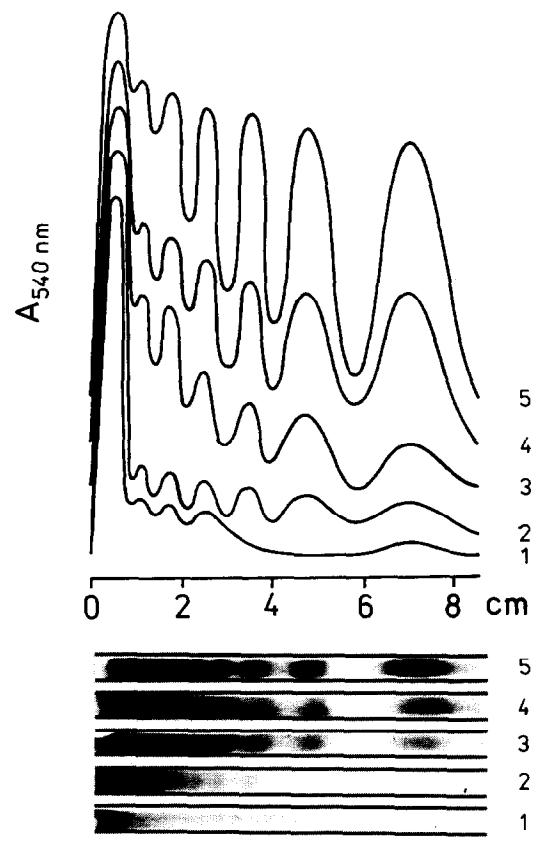

Fig.1. Electrophoretic separation of DNA isolated from thymus nuclei of normal mice (1), from thymus nuclei of mice killed 1, 2 and 4 h after 600 R whole-body irradiation (2, 3, 4) and from salt-soluble chromatin fraction of thymuses from mice killed 6 h after irradiation (5).

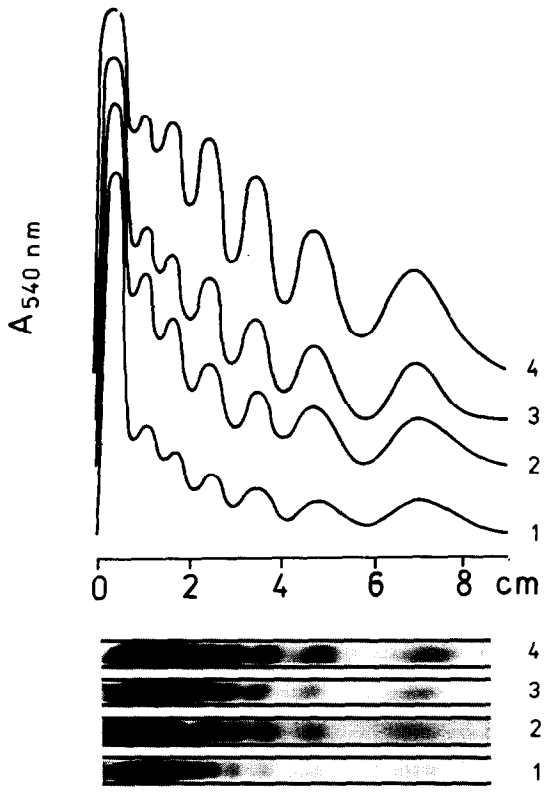

Fig.2. Electrophoretic separation of DNA isolated from thymus nuclei of normal mice digested by 7.5 U micrococcal nuclease at 37°C for 1, 2.5, 5 and 10 min (1–4).

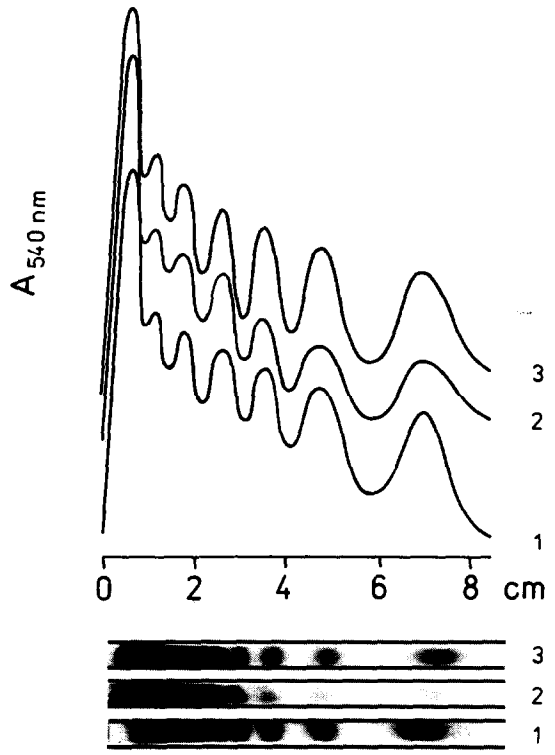

Fig.3. Electrophoretic separation of DNA isolated from thymus nuclei 4 h after 600 R whole-body irradiation (1), of DNA isolated from nuclease-digested (7.5 U, 5 min) thymus nuclei (2) and of a mixture of both samples (3).

chromatin from mice 6 h after irradiation (gel and scan No. 5 in fig.1). DNA isolated from spleens of irradiated mice gives a pattern similar to thymus DNA. Contrary to this, DNA isolated from liver nuclei of irradiated mice shows no regular distribution: some material is, however, found as a broad band with high mobility (results not shown).

3.2. *Comparison of DNA fragments obtained from nuclease-digested nuclei and nuclei from irradiated tissues*

The digestion of nuclei isolated from mouse tissues (thymus, spleen, liver) by micrococcal nuclease causes degradation of chromatin into subunits with the typical DNA electrophoretic pattern. The densitometric scans of DNA isolated from digested thymus nuclei are shown in fig.2; spleen and liver nuclei give similar results.

For the sake of comparison, DNA samples isolated from nuclease-digested (5 min) thymus nuclei and from thymus nuclei of mice 4 h after irradiation, were co-electrophoresed. As seen in fig.3, the migration of

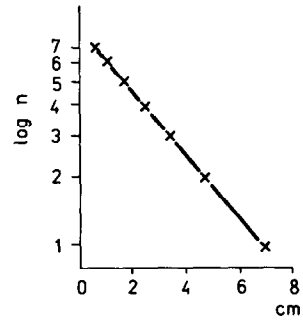

Fig.4. Plot of logs of band numbers versus mobility in 4% PAAG for DNA samples from fig.3.

discrete DNA classes is identical in both DNA samples. If the mixture of both DNA samples. If the mixture of both DNA samples is subjected to electrophoresis (gel and scan No. 3 in fig.3) the peaks are well preserved and no spreading of the bands occurs. This shows that the size of DNA molecules in different classes of both samples is the same. Moreover, the linear relationship between the logs of band numbers and electrophoretic mobility (fig.4) proves that the size of DNA fragments is a multiple of the unit length.

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