On the Role of Ca, Mg-dependent Nuclease in the Postirradiation Degradation of Chromatin in Lymphoid Tissues

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Abstract. The characteristics of the postirradiation degradation of chromatin in thymocytes in vivo were compared with the features of chromatin fragmentation in isolated thymocyte nuclei in vitro by endogenous chromatin-bound nucleases. Nuclease which degrades chromatin produces in vivo fragments of nucleosomal size; the double-strand breaks appear as the result of the accumulation of single-strand breaks with 3'-OH ends; the nuclease is inhibited by Zn^{2+} and DTNB and its activity is depressed by cycloheximide pretreatment. In experiments on in vitro degradation of chromatin in isolated thymocyte nuclei similar properties were observed for the Ca, Mg-dependent, but not for acid nuclease. The results bring further evidence of the involvement of an enzyme of the Ca, Mg-dependent nuclease-type in chromatin degradation in irradiated thymocytes.

Key words: Chromatin degradation — Thymocytes in vivo — Nuclei in vitro — Chromatin-bound nucleases — Inhibitors

Introduction

It is well established that postirradiation damage of lymphoid cells is accompanied by internucleosomal degradation of the nuclear chromatin (Skalka et al.

Abbreviations: DTNB — 5,5'-dithio-bis (2-nitrobenzoic acid),
EDTA — ethylene diamine tetraacetic acid,
PMSF — phenylmethyl sulfonylfluoride,
Tris — tris(hydroxymethyl)-aminomethane

1976; Zhivotovsky et al. 1980; Umansky et al. 1981; Matyášová et al. 1984). An analogous chromatin degradation is observed also in lymphoid cells dying under the effect of glucocorticoids (Wyllie 1980; Umansky et al. 1981; Cohen and Duke 1984; Ucker 1987) as well as during an incubation of isolated thymic cells in vitro (probably related to suboptimal culture conditions). Other authors described the degradation of nuclear genetic material in the course of programmed cell death in embryogenesis (Manes and Menzel 1982), during the terminal differentiation of some cell types (Appleby and Modak 1977; Soldatenkov et al. 1985), in target cells under the effect of cytotoxic lymphocytes (Russel et al. 1982; Duke et al. 1986; Schmid et al. 1986; Ucker 1987), as well as in various cells due to the action of a number of damaging factors of physical, chemical or biological nature (Liepins and Younghusband 1985; Soloff et al. 1987; Vedeckis and Bradshaw 1983; Matyášová et al. 1979; Ermolaeva et al. 1986; Villeponteau et al. 1986; Dealtry et al. 1987).

In most cases chromatin degradation is an early event in the processes leading to cellular death. Many authors consider chromatin degradation as the result of an active cellular response, triggered by an internal signal or by an external attack (Hanson 1979; Wyllie et al. 1980; Umansky 1982; Ucker 1987). One can, therefore, understand the great effort spent by different groups (Skalka et al. 1981; Nikonova et al. 1982; Duke et al. 1983; Muel et al. 1986; Compton and Cidlowski 1987) to investigate the nature of the enzymic factor involved in the chromatin scission in cells destined to death. Indirect indices obtained from various experiments have suggested the possible role of Ca, Mg-dependent nuclease in this process. However, no direct evidence in this respect has been provided so far.

In the present work we compare the characteristics of postirradiation in vivo chromatin degradation in thymocytes with the features of in vitro chromatin fragmentation by the endogenous, chromatin-bound nucleases in isolated thymocyte nuclei. The results obtained provide furher evidence in favour of the role of Ca, Mg-dependent nuclease in chromatin degradation in dying lymphoid cells.

Materials and Methods

The experiments were performed in parallel in two collaborating laboratories using both rats (Wistar, males 150—180 g of weight) and mice (F1 hybrids (CBA × C57BL/10), females, 6—8 weeks old). This experimental design allowed also a comparison of the thymic responses of the two species. Only few differences were observed and they will be discussed later.

The animals were whole-body irradiated from ^{60}Co sources: rats received 10 Gy (6.92 Gy/min), mice 6 Gy (0.5 Gy/min).

Isolation and short-term culture of thymocytes. Cell suspensions were prepared by pressing the thymi through a plastic mesh screen and suspending in Tris-buffered saline of the following final composition (in mmol.l^{-1}): Tris-HCl 10, pH 7.4, KCl 6, NaCl 130, $CaCl_2$ 3, 0.15 % BSA. The cells were washed in the same medium without BSA and resuspended in the incubation medium of the following composition (in mmol.l^{-1}): Tris-HCl 10, pH 7.4, KCl 6, NaCl 130, $CaCl_2$ 1, $MgCl_2$ 3. The cell suspensions (6.2×10^7 cells per 2 ml medium) were incubated in siliconized glass probes in an atmosphere with 5—8 % CO_2 on a roller at 37 °C for 0—6 h. At the end of the incubation the medium was washed out and the DNA degradation products were extracted by homogenization of the cells in a solution containing 140 mmol.l^{-1} NaCl and 15 mmol.l^{-1} sodium citrate. After centrifugation (2500 × g, 10 min) the DNA content was estimated both in the supernatant and in the sediment by means of diphenylamine reaction.

Thymocyte nuclei were isolated in the solution containing 250 mmol.l^{-1} sucrose, 3 mmol.l^{-1} $MgCl_2$, 10 mmol.l^{-1} Tris-HCl, pH 7.2. The nuclei were washed three times in the same solution (800 g, 10 min) and finally resuspended in 10 mmol.l^{-1} Tris-HCl, pH 7.2 and 3 mmol.l^{-1} $MgCl_2$. In some experiments mouse thymocyte nuclei were isolated in solutions with Ca^{2+} substituted for Mg^{2+} ions (5 mmol.l^{-1} Tris-HCl, pH 7.2, 5 mmol.l^{-1} $CaCl_2$ in 250 mmol.l^{-1} sucrose solution and 10 mmol.l^{-1} Tris-HCl, pH 7.2, 1 mmol.l^{-1} $CaCl_2$ in washing solution). PMSF (1 mmol.l^{-1}) was added to all the above solutions.

DNA was isolated and purified from rat thymocyte nuclei according to the method of Maniatis et al. (1982). 3'-OH ends in DNA were estimated by the nick-translation reaction 5'-OH ends by a direct polynucleotide-kinase reaction, both according to the description of Maniatis et al. (1982). The radioactivity of the reaction products was measured using a scintillation counter SL-30.

DNA digestion with Sl nuclease. The isolated DNA was incubated in a medium containing 30 mmol.l^{-1} sodium acetate, pH 4.6, 50 mmol.l^{-1} NaCl, 1 mmol.l^{-1} $ZnCl_2$ and 150 U of the enzyme. The reaction was stopped by adding SDS to a final concentration of 2 %.

For studies of in vitro degradation by endogenous nucleases the isolated nuclei were incubated (600 μg DNA/ml) at 37 °C in the following media:

I. 50 mmol.l^{-1} Tris-HCl, pH 7.5, 60 mmol.l^{-1} KCl, 15 mmol.l^{-1} NaCl, 1.5 mmol.l^{-1} 2-mercaptoethanol, 1 mmol.l^{-1} EDTA, 10 mmol.l^{-1} $MgCl_2$, 1 mmol.l^{-1} $CaCl_2$ (for Ca, Mg-dependent nuclease);

II. 50 mmol.l^{-1} Tris-HCl, pH 7.5, 60 mmol.l^{-1} KCl, 15 mmol.l^{-1} NaCl, 1.5 mmol.l^{-1} 2-mercaptoethanol, 1 mmol.l^{-1} EDTA, 10 mmol.l^{-1} $MgCl_2$ (for Mg-dependent nuclease);

III. 50 mmol.l^{-1} imidazole-HCl, pH 5.4, 60 mmol.l^{-1} KCl, 15 mmol.l^{-1} NaCl, 1.5 mmol.l^{-1} 2-mercaptoethanol, 2 mmol.l^{-1} EDTA (for acid nuclease); in experiments with mouse thymocyte nuclei this medium was prepared with sodium acetate buffer, pH 5.4, instead of imidazole;

IV. 10 mmol.l^{-1} Tris-HCl, pH 7.5, 1.5 mmol.l^{-1} 2-mercaptoethanol, 1 mmol.l^{-1} EDTA, 10 mmol.l^{-1} $MgCl_2$, 1 mmol.l^{-1} $CaCl_2$ (low ionic strength medium for Ca, Mg-dependent nuclease).

The reaction was stopped by chilling and adding EDTA to a concentration of 10 mmol.l^{-1} (for media I, II and IV) or $MgCl_2$ to the same concentration (for medium III). The suspension was centrifuged at 2500 × g for 5 min and the supernatant was discarded (as it was found to contain no appreciable amounts of DNA). From the sedimented nuclei fragments of chromatin were extracted with 0.7 mmol.l^{-1} EDTA solution, pH 7.2, and isolated by centrifugation (22000 × g, 25 min). The DNA content in supernatants and sediments was determined upon hydrolysis in 0.5 mol.l^{-1} $HClO_4$ either by direct spectrophotometry or by diphenylamine reaction.

DNA isolation for electrophoresis. Suspensions of nuclei or cells were supplemented with NaCl (to 1 mol.l^{-1}), EDTA (to 2.5 mmol.l^{-1}) and SDS (to 1 %) and cell samples additionally with Triton

X-100 (to 0.5 %); DNA was then deproteinized using chloroform-isopentanol (24 : 1). DNA was precipitated from the water layer by two volumes of ethanol (−20 °C, overnight). Electrophoresis of DNA in native conditions was performed in 3 % PAG — 0.5 % agarose or 1 % agarose (Maniatis et al. 1975). The denatured DNA was electrophoresed in 6 % PAG according to Simpson and Whitlock (1976). The gels were stained by ethidium bromide or Stains-all.

Inhibitors. $ZnSO_4$: The nuclei were incubated in media as above and 100 μmol . l^{-1} $ZnSO_4$ were added. For cells the concentration was raised to 1 mmol . l^{-1}.

5,5'-dithio-bis/2-nitro/benzoic acid (Pierce Chemical Co., Rockford, Il., USA) was neutralized and used as sodium salt. The nuclei were preincubated with 2 mmol . l^{-1} DTNB in 10 mmol . l^{-1} Tris-HCl, pH 7.2, 1 mmol . l^{-1} $CaCl_2$ for 15 min, and then washed out with the same solution without the inhibitor; finally they were incubated in the media as above. Cell suspensions were incubated in the presence of 2 mmol . l^{-1} DTNB.

Cycloheximide: Rats were injected i.p. with 300 µg cycloheximide (Koch-Light Lab.) /100 g of body weight.

Results

I. Characteristics of in vivo chromatin degradation

The degradation of chromatin to salt-soluble fragments occurs in mouse and rat thymi and spleens as soon as after 2 h following irradiation, and reaches maximum at 6—8 h (Cole and Ellis 1957; Skalka et al. 1965; Umansky et al. 1981). Although the extent of fragmentation increases with the radiation dose up to about 3 Gy (Skalka and Matyášová 1963), the onset and kinetics of chromatin degradation are almost independent of the radiation dose (Umansky et al. 1981; Pechatnikov et al. 1986). Electrophoretic analysis of the degradation products revealed discrete bands, corresponding to nucleosomes and their oligomers. Native DNA isolated from the fragments corresponded in length to DNA segments obtained from the micrococcal nuclease digest of control nuclei. The amounts of mono- and oligonucleosomal DNA fragments increased with the postirradiation time, but the relative ratio of smaller and larger fragments changed only little with the postirradiation time (Fig. 1).

When the DNA isolated from the irradiated tissue was electrophoresed in denaturing conditions (Fig. 2), only fragments on nucleosomal and larger size were found; no smaller single-stranded fragments of the length of multiples of 10 nucleotides were observed which are typically found after DNaseI digestion of chromatin. This means that the degradation of chromatin in dying thymocytes does not affect the DNA of nucleosomal cores. No differences were observed in the responses of thymi between mice and rats.

One major characteristic of endonucleases is the nature of the end groups of fragments. Earlier it was proved that the products of deep degradation of

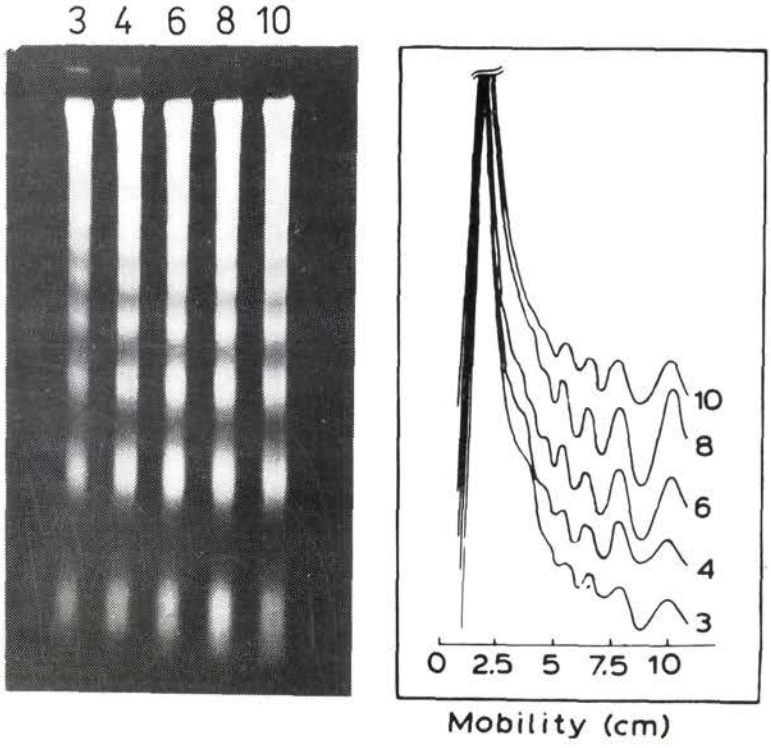

Fig. 1. Electrophoresis (3 % PAG — 0.5 % agarose) of native DNA isolated from thymocytes of irradiated rats (3, 4, 6, 8, 10 h after 10 Gy).

DNA in irradiated thymocytes bear 3'-OH ends (Swingle and Cole 1967). It cannot, however, be excluded that the initial attack on the genome and the final fragmentation to nucleosomes and their oligomers may be realized by various enzymes. Therefore, the character of DNA breaks were studied at early postirradiation intervals and experiments were performed aimed at studying the mechanism of double-stranded breaks, in particular to show whether double-strand breaks appeared as the result of the accumulation of single-strand breaks. The 3'-OH ends were determined by means of nick-translation, i. e. a reaction allowing to detect only single-strand breaks bearing 3'-OH ends. The 5'-OH ends were determined by means of a polynucleotide-kinase reaction which detects these ends of single- and double strand breaks. As apparent from Fig. 3, irradiation immediately induces DNA breaks bearing both 3'-OH and 5'-OH ends. After 60 min postirradiation, the repair of initial DNA damage is almost completed and the frequencies of both break types return to the control values. This fully corresponds to data on the DNA-repair kinetics in thymocytes

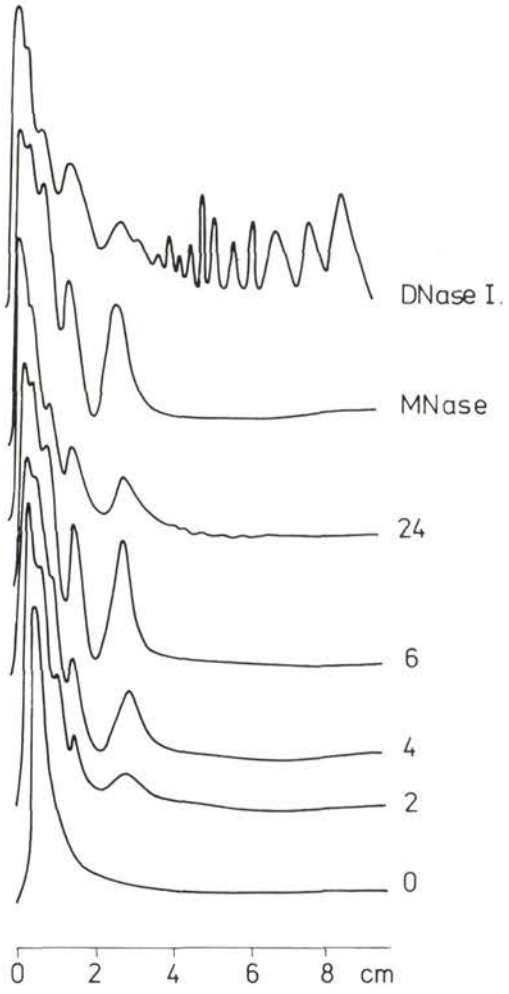

Fig. 2. Densitograms of electrophoretic separation (6 % PAG) of denatured DNA isolated from thymocytes of irradiated mice (0, 2, 4, 6, 24 h after 6 Gy) compared with DNA isolated from control nuclei after slight digestion with MNase and DNase I.

of irradiated animals, obtained by other methods (Scaife 1972; Ono and Okada 1974; Ivannik et al. 1975). Subsequently (studied up to 2 hours after irradiation) the amounts of 3'-OH ends start increasing, while those of 5'-OH ends remain unchanged. The results show that chromatin degradation starts with the occurrence of single-strand breaks with 3'-OH ends, localized in internucleosomal linker segments.

To confirm this, in further experiments the DNA isolated from thymocytes

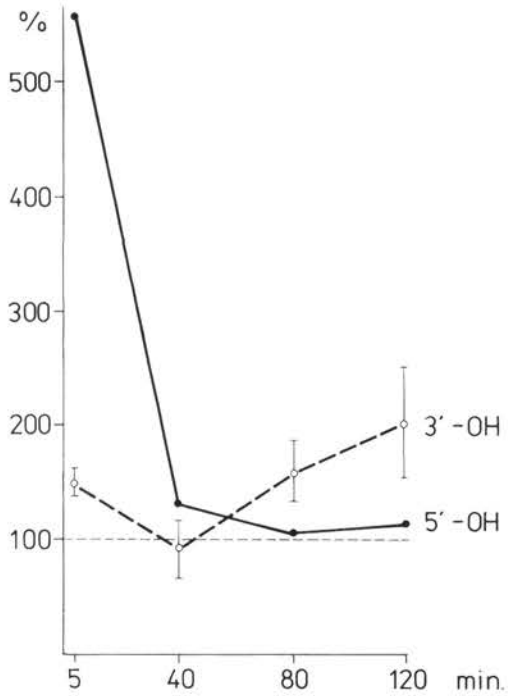

Fig. 3. The amounts of 3'-OH and 5'-OH ends in DNA isolated from thymocytes of irradiated rats. *Abscissa*: Time after irradiation (10 Gy); *Ordinate*: Label incorporation is expressed as per cent of the values measured in DNA of control animals (dashed line).

2 h after irradiation of the rats was incubated with nuclease Sl under conditions allowing digestion of DNA only in single-stranded regions. The electrophoretogram shown in Fig. 4 suggests that, upon digestion with Sl nuclease, a portion of the DNA has been degraded to fragments of oligonucleosomal size, while the DNA from the undigested sample still moves as a single, macromolecular band.

Thus the results show that the enzymatic degradation of chromatin in thymocytes of irradiated animals starts only after the initial radiation damage to DNA has been almost fully repaired. Later on single-strand DNA breaks with 3'-OH ends start accumulating in the internucleosomal linker segments. Finally, this leads to chromatin degradation to nucleosomal oligomers. Their DNA is saved from single-strand breaks even at later intervals.

II. Characteristcs of chromatin degradation in isolated nuclei

The question arises, which of the nucleases, present in the chromatin, is able to

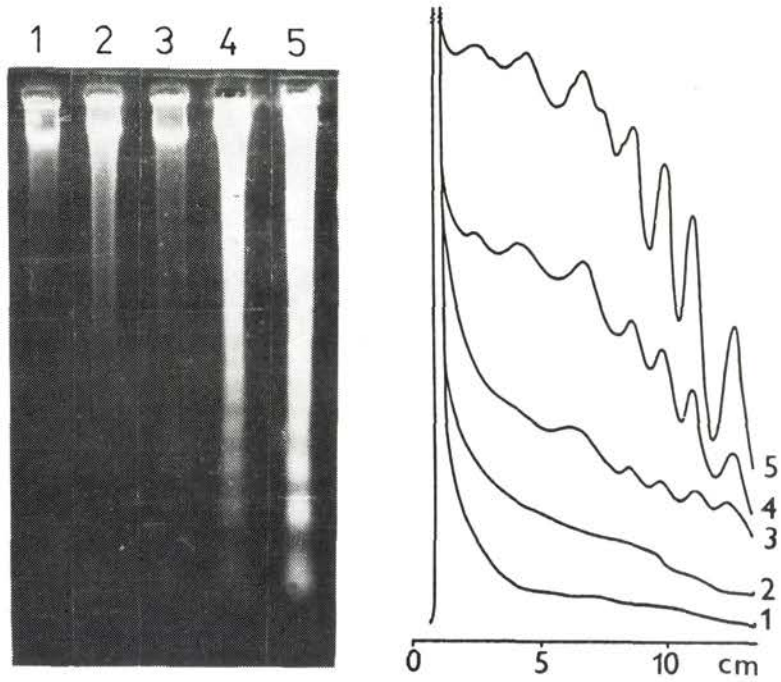

Fig. 4. Electrophoresis (1 % agarose) of native DNA isolated from thymocytes of control and irradiated rats and digested with Sl nuclease; 1,2 — DNA from control animals without and with additional Sl nuclease digestion; 3, 4 — DNA from irradiated animals (2 h after 10 Gy) without and with additional Sl nuclease digestion; 5 — DNA from irradiated animals (6 h after 10 Gy) without Sl nuclease digestion.

cause chromatin fragmentation analogous to that observed *in vivo* in irradiated cells. Isolated nuclei from thymocytes were incubated in various media and digestion products were characterized.

At least two endogenous nucleases are present in the nuclei (Fig. 5), which are able to split DNA in linker segments; Ca, Mg-dependent nuclease (Hewish and Burgoyne 1973) and a nuclease acting at acidic pH and not requiring divalent cations. Mg-dependent nuclease found in nuclear extracts (Nikonova et al. 1982) is, in rat thymocyte nuclei, less active in cleaving chromatin DNA.

Neither acid endonuclease nor the Ca, Mg-dependent nuclease form substantial amounts of intranucleosomal scissions even after prolonged action at physiological ionic strength (Fig. 6). However, in low ionic strength solution the Ca, Mg-dependent nuclease cleaves DNA also in the nucleosomal core, thus

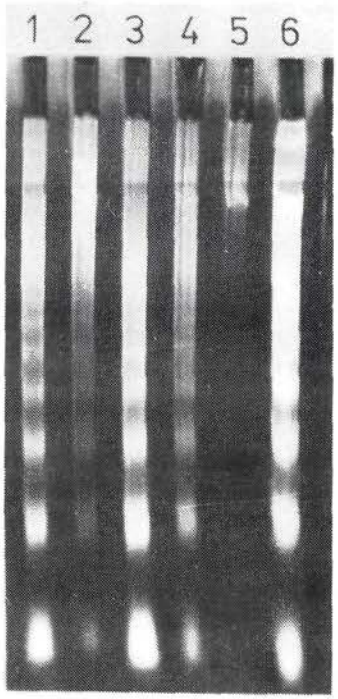

Fig. 5. Electrophoresis (3 % PAG — 0,5 % agarose) of native DNA isolated from rat thymocyte nuclei incubated for 30 min: 1,2 — in Ca^{2+} and Mg^{2+} containing medium (10 μg DNA per lane); 3, 4 — in acidic medium (10 μg and 5 μg DNA per lane); 5 — in Mg^{2+} containing medium (5 μg); 6 — compared with native DNA (20 μg) isolated from thymocytes of in vivo irradiated rats (6 h after 10 Gy).

resembling in its action DNase I. This effect may be connected with decondensation of chromatin in low ionic conditions (Matyášová and Skalka 1986).

Some differences in the relative activities of the enzymes between murine and rat thymocyte nuclei should be mentioned. In rat nuclei the acid enzyme is more active than the Ca, Mg-dependent nuclease. In murine thymocyte nuclei the most active is the Ca, Mg-dependent enzyme, whereas the acid nuclease is less active, and some fragmentation is observed also in media containing only Mg^{2+} ions.

The analysis of the end groups of DNA fragments formed by nuclear enzymes shows (Fig. 7) that 3'-OH ends occur in chromatin cleaved by Ca, Mg-dependent nuclease and 5'-OH ends in chromatin cleaved by acid endonuclease (which is thus similar to the action of DNase II).

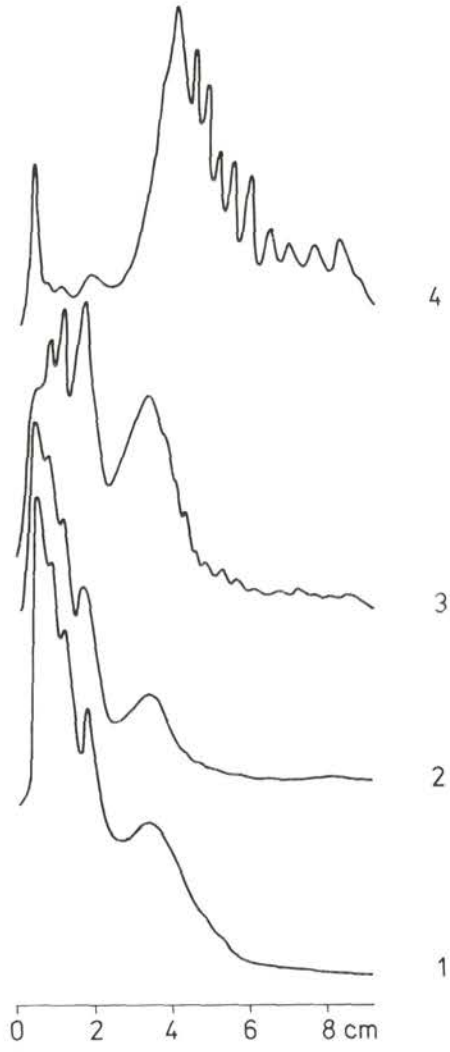

Fig. 6. Densitograms of electrophoretic separation (6 % PAG) of denatured DNA isolated from murine thymocyte nuclei incubated for 180 min: 1 — in acidic medium; 2 — in Mg^{2+} containing medium; 3 — in Ca^{2+} and Mg^{2+} containing medium; 4 — in Ca^{2+} and Mg^{2+} containing medium of low ionic strength.

The results suggest that only Ca, Mg-dependent nuclease in a medium of physiological ionic strength degrades chromatin DNA in isolated nuclei in a manner similar to the fragmentation occuring in vivo in irradiated thymocytes.

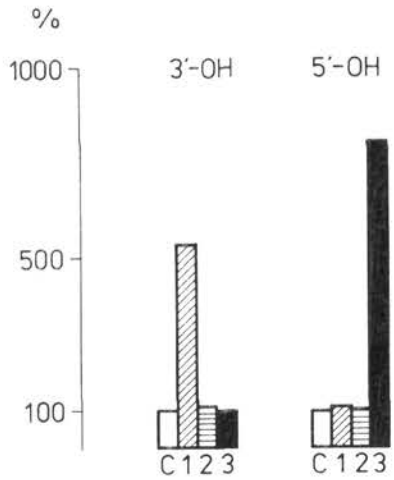

Fig. 7. The amounts of 3'-OH and 5'-OH end groups in DNA isolated from rat thymocyte nuclei incubated for 30 min: 1 — in Ca^{2+} and Mg^{2+} containing medium; 2 — in Mg^{2+} containing medium; 3 — in acidic medium; label incorporation is expressed in per cent of the values measured for control nuclei (C).

III. The effect of inhibitors on chromatin degradation by endogenous nucleases in cells and isolated cell nuclei

Cohen and Duke (1984) have shown that DNA degradation induced in thymocytes by glucocorticoids as well as that induced in target cells by cytotoxic lymphocytes (Duke et al. 1983; Dealtry et al. 1987) is inhibited in the presence of Zn^{2+} ions.

In our experiments the effect of Zn^{2+} ions on chromatin degradation was studied during in vitro incubation of thymocytes isolated from normal and irradiated mice (3 h after 6 Gy), and on in vitro chromatin degradation in nuclei. The degradation in incubated cells was fully inhibited by the presence of Zn^{2+} ions both in control cells (degrading chromatin spontaneously) and in cells isolated from irradiated mice. In incubated nuclei the action of Ca, Mg-dependent nuclease was also fully depressed; the acid nuclease was substantially less affected by Zn^{2+} ions. In subsequent experiments another inhibitor, DTNB, was used. This compound was reported to inhibit the Ca, Mg-dependent nuclease (Burgoyne and Skinner 1981). This inhibitor suppressed both the degradation of chromatin in incubated cells and the Ca, Mg-dependent activity in isolated nuclei; less pronounced was the inhibition of acid nuclease (Fig. 8).

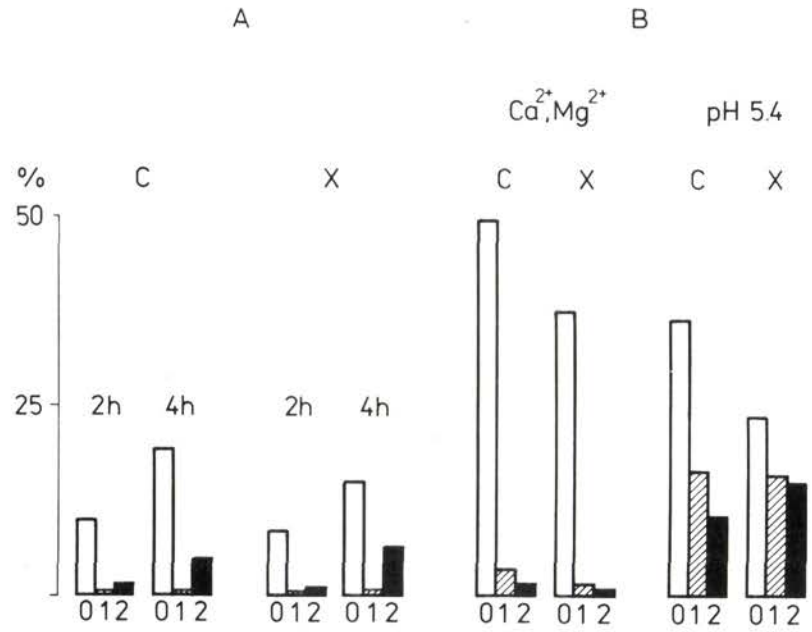

Fig. 8. The effect of Zn^{2+} ions and DTNB on chromatin degradation in isolated cells and nuclei from normal (C) and irradiated (X) mice (3 h after 6 Gy) and incubated in vitro: *A*-degradation in thymocytes incubated for 2 and 4 hours: 1 — with 1 mmol.l^{-1} $ZnSO_4$; 2 — with 2 mmol.l^{-1} DTNB; the degree of degradation is expressed as percentage of saline-soluble DNA (the values of degradation products found in parallel samples without incubation were subtracted). *B*-degradation in murine thymocyte nuclei incubated for 30 min in medium containing Ca^{2+} and Mg^{2+} or in acidic medium (pH 5.4): 0 — without inhibitor; 1 — with 100 μmol.l^{-1} $ZnSO_4$; 2 — nuclei preincubated with 2 mmol.l^{-1} DTNB; the degree of degradation is expressed as percentage of DNA solubilized by 0.7 mmol.l^{-1} EDTA solution (the values of degradation products found in parallel nonincubated samples were subtracted).

Several authors (Ermolaeva and Vodolazskaya 1977; Ivannik et al. 1978) have observed that chromatin fragmentation in thymocytes of irradiated animals was depressed by inhibitors of protein synthesis. We now performed experiments in which the activity of endogenous nucleases in thymocyte nuclei, isolated from control and irradiated rats was studied at various intervals after cycloheximide injection. The results (Fig. 9) show that the activity of acid nuclease is not changed by cycloheximide treatment. On the other hand, the activity of Ca, Mg-dependent nuclease declines and no activity can be recorded in nuclei isolated from both control and irradiated animals 3 h after cycloheximide injection. A similar decrease in the activity of Ca, Mg-dependent nuclease was previously observed by Yamamoto et al. (1984) in liver cell nuclei of

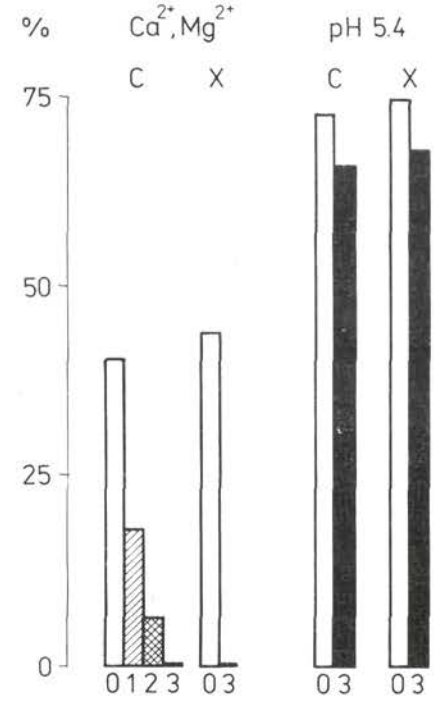

Fig. 9. The effect of cycloheximide pretreatment on chromatin degradation in nuclei isolated from thymocytes of control (C) and irradiated (X) rats 3 h after 10 Gy; the nuclei were incubated in medium containing Ca^{2+} and Mg^{2+} ions or in acidic medium (pH 5.4) for 30 min: 0 — nuclei isolated from rats without cycloheximide treatment; 1, 2, 3 — nuclei from rats treated with cycloheximide (300 μg/100 g body weight) 1, 2 or 3 hours before killing; the degree of degradation is expressed as percentage of DNA solubilized by 0.7 mmol.l^{-1} EDTA solution (the values of degradation products found in parallel nonincubated samples were subtracted).

cycloheximide treated rats. It can be therefore assumed that Ca, Mg-dependent nuclease is an enzyme with a high metabolic rate.

The results of these experiments using inhibitors further support the hypothesis concerning the involvement of Ca, Mg-dependent nuclease in the process of postirradiation chromatin degradation.

Discussion

The results presented in this paper provide evidence that the nuclease(s) which

degrade chromatin in thymocytes of irradiated animals has the following properties: (1) the DNA fragments bear 3'-OH ends; (2) the double-strand breaks are formed by accumulation of single-strand breaks; (3) in nuclei the cleavage occurs only in the internucleosomal linker segments; (4) the degradation of chromatin is inhibited by Zn^{2+} ions and DTNB; (5) chromatin degradation in vitro is prevented by in vivo pretreatment with cycloheximide (this, however, is not necessarily connected with the enzyme properties alone).

The in vitro analysis of the activity of various chromatin-bound nucleases in nuclei showed that at least two endogenous enzyme species are able to degrade chromatin to nucleosomes: the acid enzyme and the Ca, Mg-dependent enzyme. The participation of the former enzyme in chromatin degradation in irradiated thymocytes can be excluded, as this nuclease does not form DNA fragments with 3'-OH ends, this enzyme is only partially inhibited by Zn^{2+} ions and DTNB, and since the pretreatment of animals with cycloheximide does not abolish the activity of the enzyme.

On the other hand, the Ca, Mg-dependent nuclease meets all the above criteria: it degrades nuclear DNA in linker segments, double-strand breaks are formed by accumulation of single-strand breaks with 3'-OH ends, the enzyme is inhibited by Zn^{2+} ions and DTNB, and pretreatment with cycloheximide depresses the activity of the Ca, Mg-dependent nuclease in thymocytes of both control and irradiated animals. Earlier results should also be mentioned: the activity of the Ca, Mg-dependent nuclease was observed to increase in the nuclei of irradiated rat cells (Nikonova et al. 1982).

Also, several results obtained with other cellular systems, in which chromatin degradation was described, suggest the role of Ca, Mg-dependent nuclease. Similarly as in irradiated thymic cells, chromatin degradation in embryonic lens fibres occurs as an accumulation of single-strand breaks (Modak and Beard 1980). As mentioned earlier, the enzyme responsible for the degradation of chromatin in glucocorticoid-treated cells and target cells is inhibited by the presence of Zn^{2+} ions (Cohen and Duke 1984; Duke et al. 1983; Dealtry et al. 1987) and thus resembles the Ca, Mg-dependent nuclease. Recently, increased activity of the Ca, Mg-dependent nuclease(s) in thymocytes of glucocorticoid-treated rats was directly proved by Compton and Cidlowski (1987).

A number of questions concerning the proper mechanism leading to nuclease activation in the nuclei of various cell types under various conditions remains unclear. Are the trigger mechanism and the activation processes identical in all the mentioned cell types? Is the process based on the activation of a pre-existing nuclease, already present in the nuclei, or does a new, similar nuclease need to be synthesized do novo? Most cases of chromatin degradation (with the exception of cell-mediated lysis) seem to require new protein synthesis,

though only recently new enzyme species were described in thymocytes upon glucocorticoid treatment (Compton and Cidlowski 1987).

Several possibilities have been considered for the activation of the pre-existing nuclease. One of them could be the decreased level of the naturally occuring nuclease inhibitor in irradiated rats, as suggested by Promwichit et al. (1982). Another possibility can be decreased poly ADP-ribosylation of proteins, since this modification depresses the activity of the Ca, Mg-dependent nuclease (Yoshihara et al. 1975); indeed, a marked decrease in the poly (ADP-ribose) polymerase activity (preceding chromatin degradation) was observed in the thymocytes of irradiated rats (Zotova et al. 1983; Nelipovich et al. 1985). Undoubtedly, an important role in the activation of the Ca, Mg-dependent nuclease can be played by changes of ionic homeostasis and by structural alterations of chromatin, which can facilitate access for the nuclease. So, we have shown earlier that several antigenic determinants in chromatin are better accessible to antibodies already 2 h after irradiation (Zotova et al. 1985), suggesting chromatin decondensation.

It can be summarized that all of the available data suggest the role of Ca, Mg-dependent nuclease in chromatin degradation in irradiated thymocytes. Nevertheless, the data available are insufficient to allow conclusive description of the exact way leading to activation of the endogenous enzyme(s) or to prove the need for synthesis of new enzyme(s), or finally, to exclude the participation of some other pre-existing or newly synthesized biocatalytic factor(s), at least in the initial stage of the process. Conclusive answers to all the questions concerning genome degradation in thymocytes and in other cellular systems await further comparative experiments.

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