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*Letter to the Editor*

# Differences in Cleavage of Untreated and Adriamycin-treated Chromatin from Normal and Leukemic Human Cells by Site Non-specific Endoribonucleases

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INVESTIGATIONS on the accessibility of DNA in chromatin to site non-specific DNases have been useful for the recognition of the nucleosome model for chromatin and for determining other characteristics [1]. For example, DNase I, DNase II and micrococcal nuclease (MCN) hypersensitive sites in chromatin are commonly present in the vicinity of active genes [1–5]. Non-specific DNases have also been used to examine alterations in chromatin on its interaction with DNA-intercalcating antitumor agents such as adriamycin which cause *in vivo* DNA damage and fragmentation, protein-linked DNA breaks and inhibition of both DNA and RNA synthesis [6–8].

Here we have compared the DNase I, DNase II and MCN cleavage of chromatin from normal and various human leukemic cells and the effect of adriamycin treatment on chromatin digestion by the above enzymes to elucidate differences in above cells and study the effect of drug on conformational changes in chromatin.

Unstimulated and 72-hr phytohemagglutinin (PHA) stimulated normal human lymphocytes prepared as described previously [9], cells from common acute lymphoblastic leukemia (C-ALL), B-cell acute lymphoblastic leukemia (B-ALL), acute myeloblastic leukemia (AML) and chronic lymphocytic leukemia (CLL) patients obtained by leukapheresis and containing > 90% leukemic cells were used.

Log phase cultures Ca 400 ml of Molt-4, U-937, ML-1 and K562 cell lines [T-cell ALL, histiocytic lymphoma (monoblastic), AML, and blastic phase chronic myelogenous leukemia origin respectively] grown in RPMI-1640 medium containing 5% fetal calf serum were treated with 0.5 μg/ml of adriamycin for 2 hr at 37° C. The control and drug treated cells were pelleted by centrifugation, washed with phosphate buffered saline and used for chromatin preparation. The chromatin was prepared using buffer containing ImM phenyl methyl sulfonyl fluoride and purified by ultracentrifugation through 2.2 M sucrose [9]. The chromatin was washed by suspension in 10 mM Tris–HCl, pH 7.0 and pelleting (10,000 ***g***, 15 min) and subsequently resuspended in the same buffer. To determine alterations in nuclease digestion of chromatin on adriamycin treatment, the isolated chromatin from C-ALL, B-ALL and PHA-stimulated normal lymphocytes was first treated with adriamycin as follows. Chromatin suspension (equivalent to about 2 mg DNA in 2 ml of 10 mM Tris–HCl, pH 7.4) was incubated with 600 μg of adriamycin for 15 min at 2° C and subsequently centrifuged at 10,000 ***g*** for 10 min to recover unbound adriamycin from chromatin pellet. The pellet was washed twice by resuspension in the above buffer and centrifugation. The amount of adriamycin bound to chromatin was calculated by subtracting the amount of unbound drug in the

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supernatants from the amount of drug added. This was also confirmed by eluting drug from an aliquot of chromatin (0.5 ml) with 0.1 ml 33% $AgNO_3$ and 1 ml *n*-butanol [10]. The adriamycin was estimated using spectrophotometer ($A_{480\ m\mu}$) or spectrofluorometer (excitation at 468 mμ and emission at 588 mμ) and a standard curve prepared with known concentrations of adriamycin. The digestion of chromatin with DNase I, DNase II and MCN was carried out as follows. The digestion media for DNase I contained 10 mM Tris–HCl pH 7.2, 0.1 mM Mg $Cl_2$, for micrococcal nuclease, 10 mM Tris–HCl pH 7.2, 1 mM Ca $Cl_2$ and for DNase II 10 mM Tis–HCl pH 7.0. DNase I, MCN and DNase II were normally used at 100 units/ml and chromatin at 750–1500 μg DNA equivalent per ml. After incubation at 37° C for increasing periods of time, the reactions were stopped with 7% perchloric acid–1M NaCl (final concentrations) in ice bath. The contents were centrifuged and the $A_{260}$ of the supernatants were read against zero time samples. To calculate percentage of digestion the total DNA in chromatin was determined by heating an aliquot with 7% $HClO_4$–1 M NaCl at 75° C for 45 min measuring $A_{260}$ and $A_{320}$ of the acid soluble products and taking $A_{260}$-$A_{320}$ of 20 equal to 1 mg DNA/ml.

For gel electrophoresis, aliquots of the reaction mixture were removed at various times and the reaction stopped by adding Sarkosyl to 0.4%, sucrose to 10% and bromophenol blue to 0.1% [9]. Samples (20 μl) from it were loaded onto 1% agarose gels. The electrophoresis was carried out using Buffer B (40 mM Tris–HCl, 20 mM sodium acetate, 2 mM EDTA, 18 mM NaCl, 20 mM acetic acid, pH 8.4) at 4 hr at 100 VS. The gels were stained with ethidium bromide (0.5 μg/ml) and photographed through a red filter under ultra violet light [9]. Autodigestion of chromatin was carried out in 10 mM Tris-HCl pH 7.5 1 mM MgCl 2 which showed almost no digestion.

Although chromatin from unstimulated normal lymphocytes showed somewhat less percentage digestion by DNase I (30%) and MCN (25%) as compared to chromatin from C-ALL, AML, CLL, Molt-4 and PHA-stimulated normal lymphocytes (37–45% for DNase I and 30–40% for MCN), the DNase II gave comparable digestion of chromatin (23–28%) from all above sources (data not shown). Whether this means lower transciptional activity of unstimulated normal lymphocytes (which represent non-dividing mature cells) compared to other cells as has been interpreted in other systems [1–5] would require further experimentation. It must however be added that gene activity is not always correlated with DNase hypersensitive regions in chromatin [1]. Moreover, the above difference could not be related to the leukemic process as PHA-stimulated normal lymphocytes exhibited comparable DNase I, DNase II and MCN digestion of chromatin as the chromatin from leukemic cells. Two-hour incubation of cells with 0.5 μg/ml of adriamycin resulted in much lower binding of adriamycin (8–10 μg/mg DNA in chromatin) to chromatin as compared to the adriamycin bound (200–200 μg adriamycin/mg DNA in chromatin) when incubated directly with the isolated chromatin.

The isolated chromatin from C-ALL and PHA-stimulated normal lymphocytes when pretreated with adriamycin was, however, degraded more rapidly by DNase I, DNase II and MCN as compared to untreated chromatin (Fig. 1, bottom). Greater sensitivity of chromatin to MCN digestion on binding with intercalating agent has been reported previously [11, 12]. The treatment of chromatin inside cell with adriamycin which resulted in much lower binding of drug showed only limited increase in digestion of chromatin for U-937, K-562 and PHA-stimulated normal lymphocytes (data not shown) but a clear increase for ML-1 cells compared to untreated cells (Fig. 1, top) indicating that the effect may be related to the drug concentration and characteristics of the cells. In contrast to the above results with DNase I, DNase II and MCN, the digestion of adriamycin pretreated isolated chromatin was inhibited by restriction endonucleases Ava I, Bst NI, EcoR II, Hpa II, Msp I, Mbo II, Pst I and Pvu II (data not shown). The results of this study show that interaction of chromatin with adriamycin could result in conformational changes making it more susceptible to site non-specific DNases.

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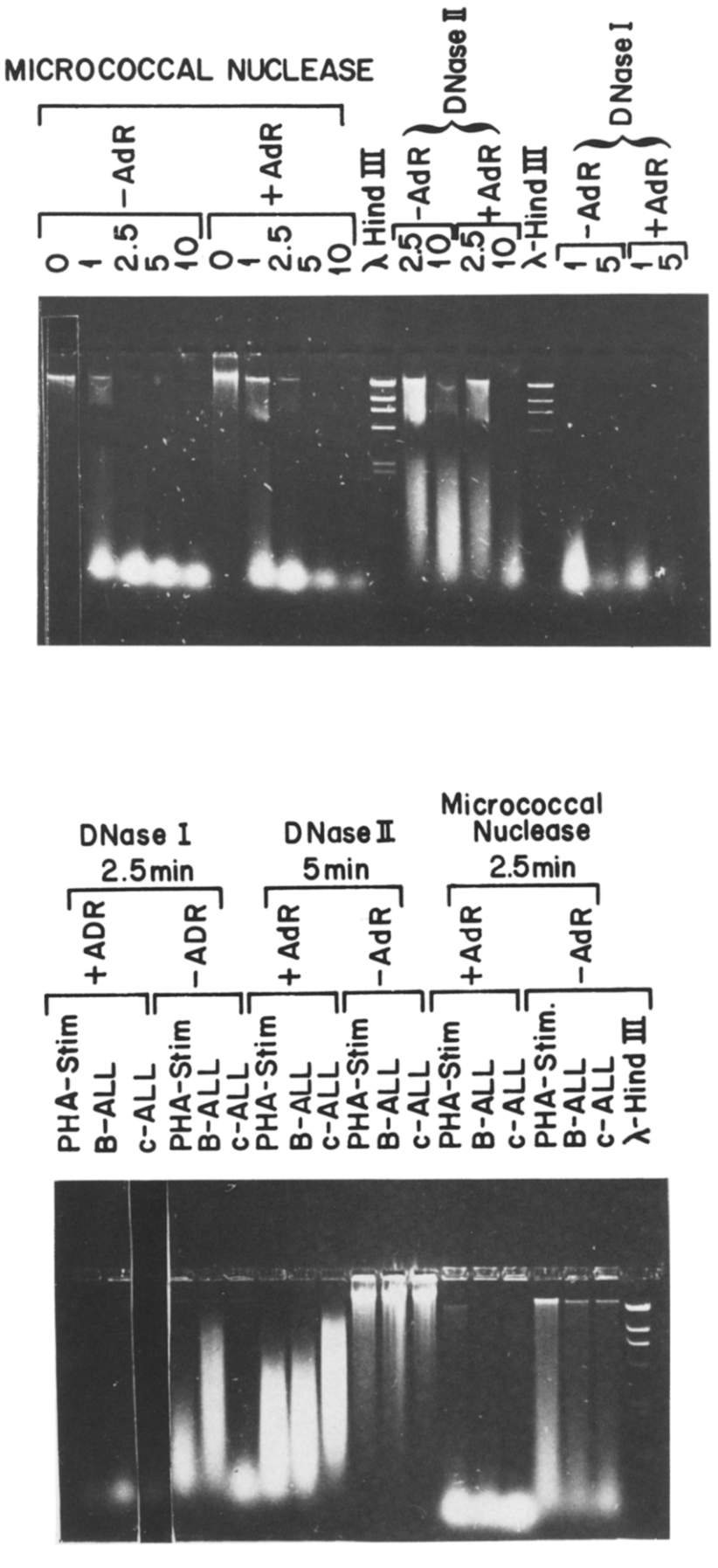


*Fig. 1. Untreated and adriamycin treated isolated chromatin from C-ALL, B-ALL and PHA-stimulated normal lymphocytes (bottom) and chromatin from untreated and adriamycin treated (0.5 μg/ml, 2 hr) ML-1 cells (top) digested at 37° C with DNase I, DNase II and MCN (1 unit/7 μg chromatin DNA) and analysed on 1% agarose gels. Zero, 1, 2.5 and 10 in top figure refer to digestion time in minutes.*

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