Alterations in the internucleosomal DNA helical twist in chromatin of human erythroleukemia cells in vivo influences the chromatin higher-order folding

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Abstract In the present study chloroquine diphosphate, a DNA intercalating drug, was used to alter the internucleosomal DNA helical twist in chromatin of living mammalian cells. The intercalative binding of chloroquine effectively unwinds the DNA double helix and its binding is restricted to nucleosomal linker regions without noticeable disruption of nucleosomes. The results presented here imply that the alterations in the rotation angle between the adjacent nucleosomes in chromatin of eukaryotic cells in vivo significantly influences the way the chain of nucleosomes folds in higher-order chromatin structures, as evidenced by specific alterations in nuclease susceptibility of chromatin.

Key words: Chromatin high-order folding; Nucleosome; Chloroquine diphosphate

1. Introduction

The packaging of DNA into the cell nucleus is a critical and often overriding factor in determining the level at which individual genes are expressed. Unraveling the mechanisms by which DNA packaging itself is controlled is therefore essential if we are to understand and usefully manipulate gene expression in eukaryotic cells. The ubiquitous basic unit of DNA packaging is the nucleosome core particle, which includes 145 bp of DNA folded into about 1.8 left-handed supercoils and wrapping around the outside of a histone octamer [1]. At the next level of organization, the nucleosomal chain winds into a helical structure stabilized by histone H1(H5) interactions to form a fiber of 20–30 nm in diameter, containing up to 8–12 nucleosomes per turn [2]. The folding of the nucleosomal fiber is particularly important because it is the folded state in which most of the chromatin is maintained during most of the cell cycle. It must be unfolded to allow transcription or replication and must be subsequently refolded to allow cell division. However, as yet we have no clear picture of how the chromatin environment changes in vivo to allow these central nuclear processes to occur. Significant advances in this area have come from the recent appreciation that chromatin fiber folding may be affected by variations in the linker DNA length between consecutive nucleosomes, giving rise to changes in the relative rotation angle as well as the distance between them [3–5].

In the present study we have used chloroquine diphosphate, a DNA intercalating drug [6,7], to alter the internucleosomal DNA helical twist in chromatin of living mammalian cells. Eukaryotic cells appear to tolerate relatively high concentrations of the drug for limited times [8,9], suggesting that it may be useful for altering DNA conformation in vivo [9]. The intercalative binding of chloroquine effectively unwinds the DNA double helix, and its binding is restricted to nucleosomal linker regions without noticeable disruption of the nucleosomes [9–11]. The results presented here imply that the alterations in the rotation angle between the adjacent nucleosomes in chromatin of eukaryotic cells in vivo significantly influences the way the chain of nucleosomes folds in higher-order chromatin structures.

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2. Materials and methods

Human erythroleukemia K562 cells were grown in RPMI 1640 medium supplemented with 10% fetal calf serum at 5% CO_2 and 37°C. At a density of 0.5–1 × 10^6 cells/ml, cells were chilled at 4°C for 30 min and chloroquine diphosphate (Sigma) was added at the desired concentrations. Cells were incubated on ice for an additional 20–30 min and glutaraldehyde solution (Sigma) was added to make 0.25% final concentration. Afterwards the cell suspensions were either incubated on ice overnight [12,13] or frozen in liquid nitrogen and left at −15°C for 3 days [14] without noticeable effect on the final result.

Ultraviolet (UV) light treatment of cells was performed under a transilluminator (312 nm, 8 mW/cm^2) for 15–20 min at 4°C. The culture dish was placed at a distance of 10 cm from the transilluminator surface.

Cell nuclei were isolated essentially as described elsewhere [13,14] except Nonidet P40 was used at 0.5–1.0% concentrations. Finally, nuclei were resuspended at 1 × 10^6 nuclei/ml in micrococcal nuclease digestion solution (50 mM Tris-HCl, pH 8.0, 100 mM NaCl, 5 mM $MgCl_2$, 1 mM $CaCl_2$, 5 mM β-mercaptoethanol, 0.05 mg/ml bovine serum albumin, 8% glycerol) and were stored at −70°C before use.

Micrococcal nuclease digestion was performed at 20°C for 15 min and was terminated by addition of an equal volume of 10 mM EDTA, 1% SDS solution containing 0.1 mg/ml proteinase K (Sigma). The mixture was incubated overnight at 37°C, DNA was precipitated by 2.5 vols. of ethanol and dissolved in gel loading buffer (10 mM EDTA, 0.1% Bromphenol blue (Reanal), 0.1% Ponco C (Reanal), 10% glycerol) containing 0.05 mg/ml of RNase A (New England Biolabs).

Agarose electrophoresis was performed in 0.6% or 1.6% agarose (Sigma) gels (as indicated in the figure legends). Gel and running buffers were TAE (40 mM Tris-acetate, 25 mM Na-acetate, 1 mM EDTA, pH 8.3) containing 0.25 μg/ml of ethidium bromide. Gels (10 × 7 × 0.3 cm) were run at room temperature at 2–5 V/cm and photographed using a 5× red filter (562 nm). Densitometer analysis of the films was done using a 300A Computing densitometer Fast Scan (Molecular Dynamics) and appropriate software.

3. Results

To examine the internucleosomal DNA spatial requirements for in vivo chromatin high-order folding we used the intercalating drug, chloroquine diphosphate, to change the linker DNA helical twist in chromatin of living human erythroleukemia K562 cells. Chloroquine is a well-studied aromatic dye that

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intercalates into DNA, causing an about 28° unwinding of the double helix at the site of intercalation [6,7]. In previous studies it was shown that chloroquine binds only to DNA, not proteins, and at low binding ratios it does not significantly alter the bending and torsional stiffness of the DNA [15,16]. Within the chromatin, chloroquine was shown to bind essentially entirely to the linker DNA [9–11] without any noticeable disassembly of nucleosome core particles [9–11]. At concentrations up to 10 mg/ml limited-time chloroquine treatment is not cytotoxic for cells [8,9], and in relatively low concentrations its effects on chromatin are limited, presumably by its intercalatory action [9].

Chloroquine at various concentrations was added to portions of K562 cell suspensions, and after 20–30 min incubation at 4°C the cells were fixed in the presence of glutaraldehyde to preserve the native chromatin structure. Nuclei isolated from fixed cells were examined for chromatin organization using micrococcal nuclease as a structural probe. Since DNA in the core of a nucleosome particle is highly resistant to micrococcal nuclease cleavage, the relative susceptibility of chromatin to micrococcal nuclease may reflect the structural peculiarities of the nucleosome chain folding. Fig. 1 shows DNA from limited nuclease digestion of nuclei from chloroquine-treated cells. At lower concentrations (0.01–0.03 mg/ml) chloroquine causes a

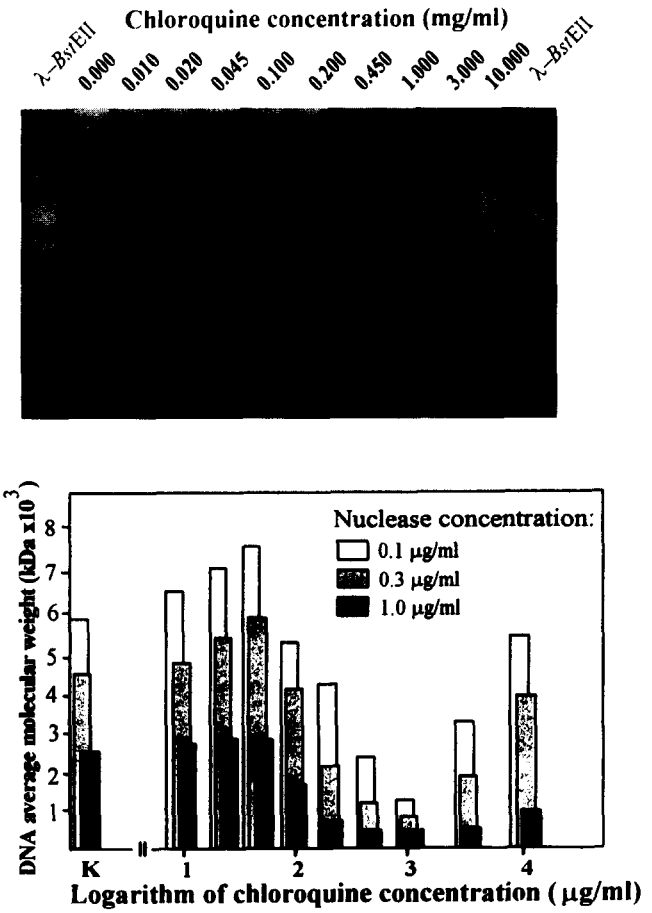

Fig. 1. Typical patterns of limited (0.1–1 μg/ml) micrococcal nuclease digestion of nuclei from cells treated with various concentrations of chloroquine diphosphate. Gel electrophoresis was performed in 0.5–0.6% agarose gels. The gel in the photograph is from an experiment using 0.3 μg/ml nuclease.

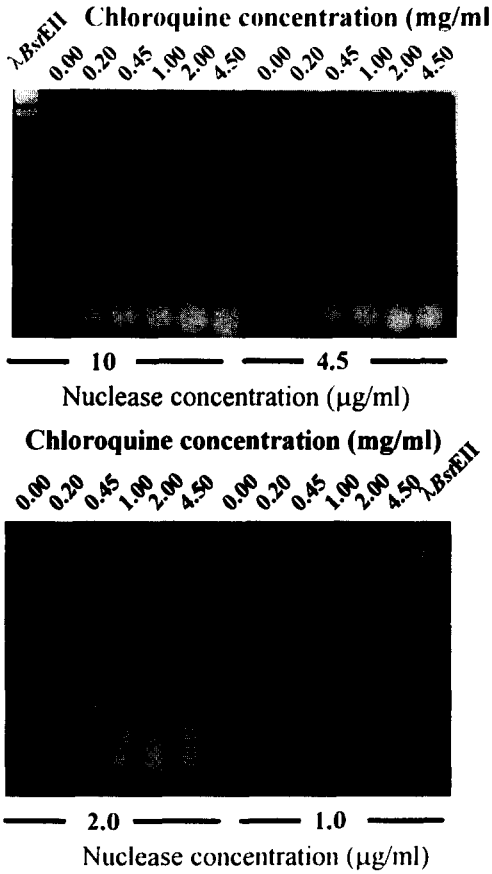

Fig. 2. Patterns of the micrococcal nuclease digestion of nuclei from cells treated with various concentrations of chloroquine. Gel electrophoresis was performed in 1.6% agarose gels.

relatively small but well-pronounced increase in the average size of digested DNA; a further increase in chloroquine concentration, up to 1 mg/ml, results in the dramatic reduction of the size of DNA fragments, and, most notably, the average size of the DNA fragments was increased again in the 1–10 mg/ml range of chloroquine concentrations, at 10 mg/ml of chloroquine the DNA fragment size becoming comparable with that of chloroquine untreated cells. Higher levels of micrococcal nuclease digestion revealed the presence of the typical nucleosome 'ladder' for all the chromatin samples (Fig. 2), suggesting that these chloroquine concentrations are probably not disrupting nucleosomes.

Experiments were performed to determine if the changes in nuclease susceptibility of chromatin of K562 cells following the addition of chloroquine are dependent on the topological closeness of DNA. Ultraviolet (UV) irradiation [18,19] and hydrogen peroxide [20,21] are well-studied DNA damaging agents that effectively introduce single- and double-strand breaks in chromosomal DNA in vivo as well as in vitro. We examined the nuclease digestion pattern in cells pre-exposed to UV light (at 312 nm) for 15 min at 4°C before chloroquine treatment. The results clearly demonstrate that the specific digestion pat-

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Fig. 4. A schematic sketch of nucleosome chain folding caused by variations in internucleosomal DNA helical twist.

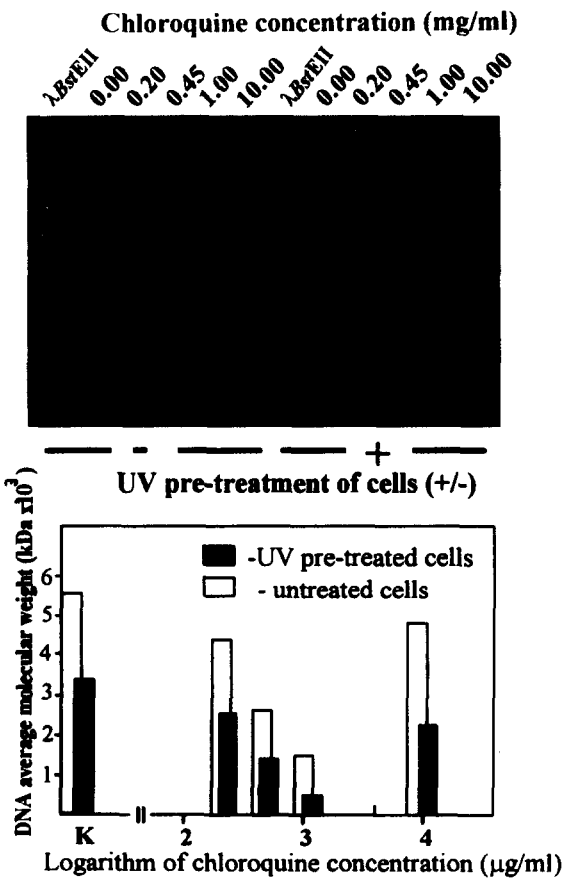
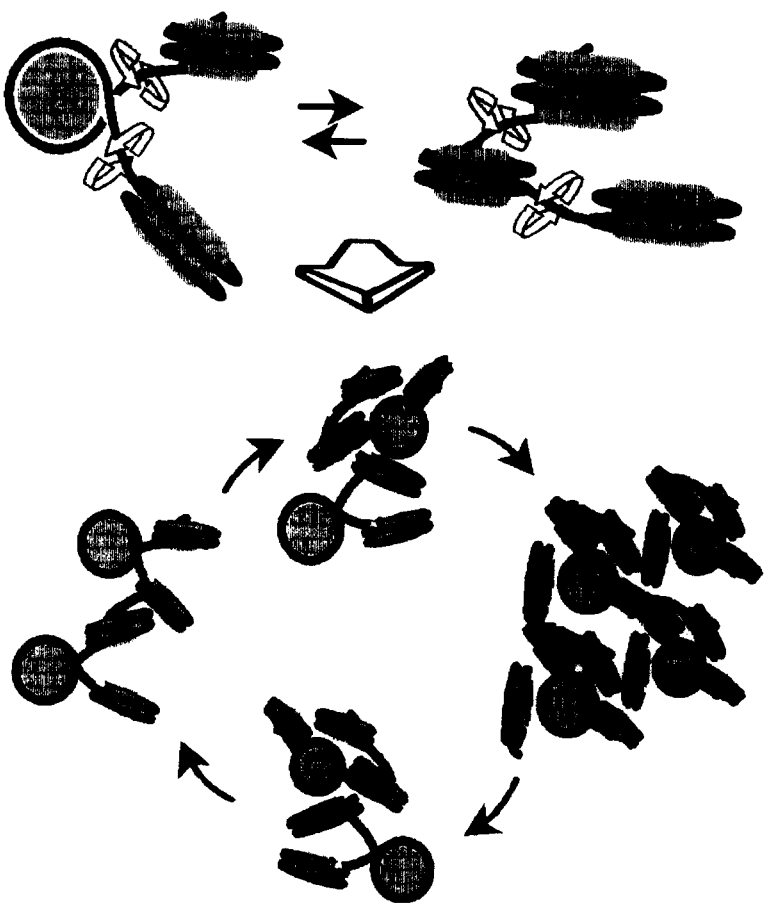


Fig. 3. Typical patterns of the limited (0.3 μg/ml) micrococcal nuclease digestion of nuclei from chloroquine-treated cells pre-exposed to UV irradiation. Gel electrophoresis was performed in 0.6% agarose gels. ←

tern is retained after UV doses of up to 30–50 kJ/m^2 (Fig. 3), although UV treatment itself results in some reduction of DNA fragment size that is presumably due to the UV-induced partial chromatin decondensation [22]. Pre-treatment of cells with 10 mM hydrogen peroxide for 30 min at 4°C had no detectable effect on the micrococcal nuclease cleavage pattern of nuclei from chloroquine-treated cells (data not shown).

4. Discussion

In the present work we have described a set of experiments using chloroquine intercalation designed to examine the effect of alterations in the integrated twist of internucleosomal DNA on chromatin folding in human erythroleukemia K562 cells. The results presented here demonstrate that the addition of chloroquine to cell suspensions gives rise to some kind of chromatin refolding processes, as demonstrated by specific alterations in the sensitivity of chromatin to micrococcal nuclease. Assuming that the observed phenomenon is a direct effect of chloroquine intercalation (see above), the obtained results imply that the way the chain of nucleosomes is folded in native chromatin in vivo is largely determined by the relative rotation angle between the adjacent nucleosomes (Fig. 4). The observed effect is not dependent on the topological closeness of DNA,



which rules out the possibility that chromatin refolding occurs due to the superhelical tension in chromatin loops induced by chloroquine intercalation.

Woodcock et al. [5] had recently theorized a range of chromatin architectures generated by continuous changes of the linker DNA length (and, therefore, the relative rotational angle) between consecutive nucleosomes. For all models a symmetrical assemblage was produced, ranging from the completely extended beads-on-a-string (relative rotational angle 180°) to fibers with the most densely packed nucleosomes (rotational angle 252–288°). The results we present here are the first evidence that such chromatin transitions may indeed take place in vivo, and that the level of chromatin folding can be 'regulated' or maintained at a defined level by variations in the helical twist of internucleosomal DNA segments.

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