ELECTRON MICROSCOPIC EVIDENCE FOR STRUCTURAL REARRANGEMENT OF H1-DEPLETED CHROMATIN DURING THERMAL DENATURATION

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1. Introduction

The possibility of histone redistribution during thermal denaturation of chromatin is a major consideration for the interpretation of melting profiles. Earlier studies based on absorbancy and CD melting are in favour of histone migration and rearrangement [1–3] but no direct evidence is available. Some data have been published which show that under certain conditions histones could dissociate from denatured chromatin to bind native DNA [3] but our earlier investigations provide evidence against histone dissociation during chromatin melting [4].

Another likely mechanism of histone redistribution is the lateral migration of histone cores along DNA. Such sliding of nucleosomes, especially after removal of histone H1, has been demonstrated after shearing [5], ultracentrifugation in high salt [6], increased ionic strength alone [7], or at 37°C [8], binding of ethidium bromide [9].

Here we present electron micrographs of partially denatured H1-depleted chromatin which show distinct rearrangement of chromatin structure. Long stretches of nucleosome-free DNA appear after chromatin melting, together with regions of closely packed nucleosomes which, at higher temperatures become clusters of dense material. This picture could be interpreted as sliding of nucleosomes during melting.

2. Materials and methods

Nuclei were isolated from rat liver [10] and chromatin was extracted after mild micrococcal nuclease digestion [11]. Histone H1 was selectively removed in 0.3 M NaCl, 0.05 M sodium phosphate buffer (pH 7) by treatment with the ion-exchange resin AG 50-X2 [12]. The H1-depleted chromatin was dialysed against 0.25 mM EDTA, pH 8. Photochemical crosslinking of DNA was performed by irradiating the chromatin with ultraviolet light of long wavelength in the presence of 8-methoxypsoralen [13].

For electron microscopy, H1-depleted samples in 0.01 M NaCl, 1 mM triethanolamine–HCl (pH 8) were fixed with 0.7% formaldehyde for 15 min and then with 0.5% glutaraldehyde for 15 min in the cold [14]. The samples (~0.5 μg DNA/ml) were applied to carbon-coated grids glow-discharged in air, or positively charged by glow-discharge in amylamine vapours [15]. In the latter case both fixed and non-fixed samples were used. The preparations were stained in 1% uranyl acetate in 70% ethanol, washed in ethanol, air-dried and rotary-shadowed with oxidized tungsten. Electron micrographs were taken at X20 000–X50 000 in a JEM 100B electron microscope. The exact magnifications were determined with a Polaron grating replica.

3. Results and discussion

H1-depleted chromatin exhibited the typical beads-on-a-string appearance of nucleosomes (fig.1) which did not change in samples crosslinked with psoralen. The linker DNA gave a broad length distribution centered around 170 Å (fig.2).

DNA in H1-depleted chromatin showed the known multiphasic melting profile in 0.25 mM EDTA-Na_3 (pH 8) with 3 well-expressed thermal transitions [16]. Crosslinking with psoralen did not much affect the melting profile but ensured renaturation of DNA even after complete denaturation. In all our experiments partially denatured samples were examined after DNA renaturation.

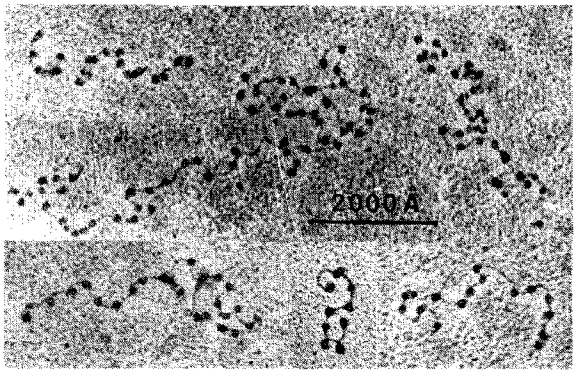

Fig.1. Electron micrographs of H1-depleted chromatin in 0.01 M NaCl, 1 mM triethanolamine, adsorbed on hydrophilic carbon grids after formaldehyde–glutaraldehyde fixation. Rotary shadowed with oxidized tungsten.

The electron micrographs of H1-depleted chromatin heated at up to 65°C (when denaturation of DNA could be reversed to 80%) showed that under our low ionic strength conditions a strong rearrangement of chromatin structure took place. Instead of the original arrangement of nucleosomes, long stretches of nucleosome-free DNA appeared together with regions of closely packed material in which individual nucleosomes could still be recognized (fig.3). This picture

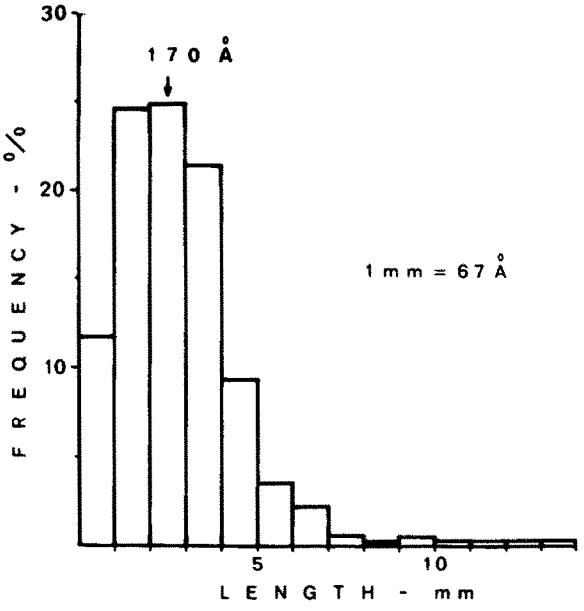

Fig.2. Histogram of linker DNA length distribution. Linker DNA length was measured on 400 internucleosomal stretches.

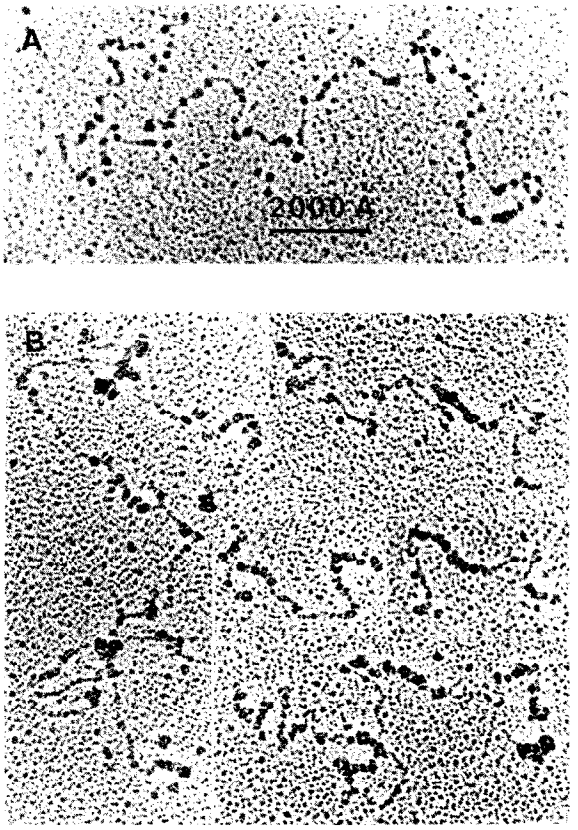

Fig.3. Electron micrographs of H1-depleted chromatin adsorbed on positively charged carbon grids before (A), and after (B) partial denaturation at 65°C. Preparations were fixed with formaldehyde–glutaraldehyde before applying to the grids. Rotary shadowed with oxidized tungsten.

could hardly be attributed to artifacts of either aldehyde fixation or adsorption to the grids since it was the same both on hydrophilic and on positively-charged carbon grids and in the latter case, both with fixed and non-fixed samples. Crosslinking with psoralen did not change the picture either.

The study of the denaturation process at >65°C was performed with H1-depleted chromatin cross-linked with psoralen. As seen in fig.4, at 72°C and 82°C (the melting temperatures of the high-temperature transitions) the electron microscopic picture of chromatin revealed stretches of nucleosome-free filaments alternating with highly condensed clusters in which no individual nucleosomes could be recognized.

Thus, our electron micrographs showed that in H1-depleted chromatin rearrangement of the nucleosomal structure occurred as early as the first thermal

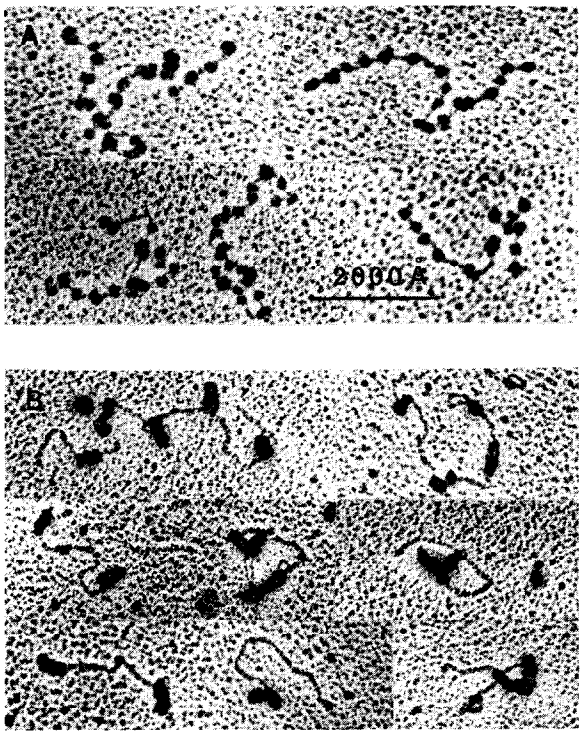

Fig.4. Electron micrographs of H1-depleted chromatin cross-linked with psoralen and adsorbed on hydrophilic carbon grids after formaldehyde-glutaraldehyde fixation: (A) control; (B) partially denatured at 72°C and 82°C. Rotary shadowed with oxidized tungsten.

transition of DNA while at higher temperatures the nucleosome morphology was lost.

The most likely explanation of the observed picture is that two different processes are involved in the different temperature ranges.

(1) A process of nucleosome sliding takes place during the low-temperature melting. This is supported by the following additional evidence obtained with partially denatured H1-depleted chromatin (in preparation):

(i) Re-denaturation of this material showed the appearance of free DNA in the melting profile;

(ii) The kinetics of its digestion with micrococcal nuclease is compatible with the presence of free DNA;

(iii) The repeat length of this material is shortened and overimposed on a high background.

It is tempting to speculate that the higher affinity of histones for doublestranded DNA could provide the driving force for nucleosome sliding, as suggested by our observation that sliding follows closely the melting of DNA. Due to the heterogeneity in the linker size (fig.2) it could be expected that DNA denaturation would begin in some longer linkers [16], thus inducing the neighbouring nucleosomes to migrate towards native DNA regions. This would further decrease the thermal stability of some DNA regions by increasing the length of unprotected DNA while other sites would become more stabilized by the shortening of the helices in h–h state [16].

(2) This process seems to be connnected with protein denaturation. The clustering of nucleosomal material which begins at about 65°C is already well expressed at 72°C. This correlates well with the temperature range of nucleosomal core protein denaturation [17]. Most likely, interactions between denatured proteins lead to aggregation of protein cores upon cooling of the samples.

A different explanation of the observed electron micrographs could be that some nucleosomes unfold in the low-temperature range of chromatin denaturation, thus accounting for the nucleosome-free regions, while other nucleosomes appear closely situated due to aggregation upon cooling. This explanation seems to us less likely in view of the biochemical data.

Whatever the mechanism may be, the structural rearrangement of H1-depleted chromatin during thermal denaturation will influence the thermal stability of different DNA regions and its dynamics must be considered in the interpretation of the melting process.

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