On the mechanism of nucleodisome splitting off by nucleases

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1. INTRODUCTION

Pancreatic DNase I is able to reveal a double-nucleosome DNA repeat in nuclei of various origins [1–3]. The resulting particle consisting of 2 nucleosomes was termed nucleodisome [2]. In the same conditions micrococcal nuclease produces DNA fragments which are multiples of the usual nucleosomal repeat length [1–3]. The modes of DNA fragmentation in chromatin by these nucleases are distinguished by at least one feature: micrococcal nuclease preferentially cleaves the linker DNA, and DNase I effectively breaks the core DNA also [4]. It seems that, besides a specific nucleosome packing in chromatin, the accessibility of certain sites of the nucleosomal DNA to nucleases can influence the formation of a double-nucleosome repeat.

To analyze the mechanism of dinucleosomal periodicity of chromatin fragmentation we used DNase II – a nuclease which is able to cleave chromatin not only in the linker DNA region (just like micrococcal nuclease), but also within nucleosome core giving rise to a 100 bp repeat [5–7]. We have demonstrated that formation of a double-nucleosome repeat generated by DNase II is the result of DNA cleavage at the sites identified within nucleosome core [7]. These sites in every second nucleosome should be relatively resistant to DNase II (and DNase I) to provide generation of the double-nucleosome periodicity.

2. MATERIALS AND METHODS

Sperm of sea urchin *Strongylocentrotus droebachiensis* was collected on the Barents Sea Marine Biological Station. Nuclei from pigeon erythrocytes and sea urchin sperm were isolated as in [8]. Washed nuclei were suspended in a solution containing 10 mM Tris–HCl buffer (pH 7.4), 3 mM $MgCl_2$, 0.1 mM PMSF for digestion with DNase I (Worthington) and in the same solution but at pH 7.0 for digestion with DNase II (HDAC, Worthington).

Electrophoresis of native DNA fragments was performed in 2% agarose gel (Sigma or BioRad) using a buffer solution containing 40 mM Tris-acetate (pH 7.8), 5 mM Na-acetate, 1 mM EDTA. Marker DNA fragments were obtained by digestion of λ-DNA with restriction endonuclease *Pst*I. The gel was stained with ethidium bromide and photographed through a red filter under ultraviolet light illumination.

3. RESULTS AND DISCUSSION

In agreement with [6,7] our results indicate that DNase II initially cleaves erythrocyte nuclear DNA at 210 bp intervals (fig.1B) and that, as digestion proceeds, the 100 bp periodicity of DNA fragmentation is revealed (fig.1C). This stage of digestion is characterized by the formation of a double-nucleosome repeat which is very similar to that found earlier after digestion of erythrocyte nuclei with DNase I ([2], fig.1E).

The 100 bp pattern of DNA fragmentation with DNase II is caused by cleavage of DNA within the nucleosome core at the sites located at 20 bp from both ends of the core DNA [7]. The distance between these sites inside the core is ~100 bp; fragments including the linker DNA are about equal in size. When chromatin with a DNA nucleosomal

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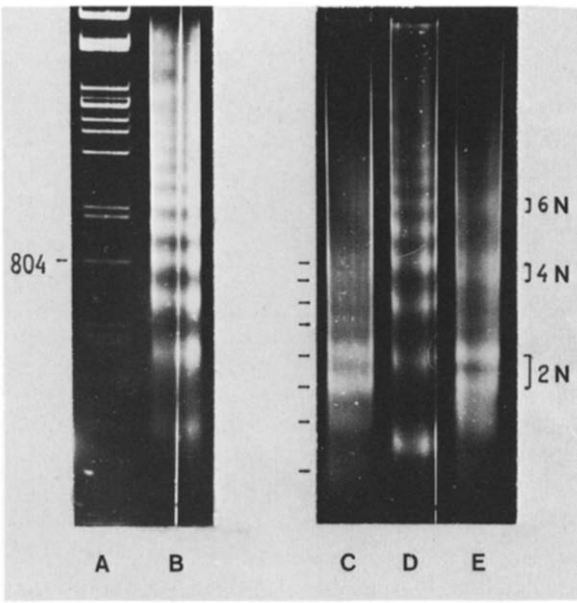

Fig.1. Electrophoresis of DNA fragments isolated from erythrocyte nuclei treated with different nucleases: (A) marker DNA fragments (λ-DNA – *Pst*I), see fig.2; (B) DNase II, initial stage of digestion (nucleosomal length DNA repeat); (C) DNase II, digestion at 100 bp intervals (the fragments of 100 bp periodicity are marked by small arrows); (D) micrococcal nuclease; (E) DNase I; 2*N*, 4*N*, 6*N*, the bands of double-nucleosome repeat.

repeat of ~200 bp is digested by DNase II a 100 bp periodicity of DNA fragmentation exactly corresponds to this value. Since DNA nucleosomal repeat in avian erythrocytes and, particularly in pigeon erythrocytes, does not much exceed this value [9], the DNA fragments produced by DNase II in the erythrocyte nuclei are multiples of ~100 bp (fig.1, [7]). These data and the results in fig.1 allow one to suppose that the dinucleosomal and the 100 bp periodicity is a result of the DNA breaks within the nucleosome core.

As was established in [7] at the stage of digestion, when 100 bp periodicity is clearly seen, the linker DNA is largely resistant to DNase II. Since DNase II produces a double-nucleosome repeat at the same stage one may conclude that during nucleodisome production linker DNA is also resistant to the nuclease. This conclusion may be checked by comparison of DNA fragments produced in a chromatin with longer linker DNA. We have used for this aim chromatin of sea urchin sperm (*Strongylocentrotus droebachiensis*) since its linker DNA is 30 bp longer than that of erythrocyte chromatin (the DNA nucleosomal repeat is equal to 240 ± 4 bp; unpublished). If DNA breaks occur at intracore sites at the stage of nucleodisome production then in the case of sea urchin chromatin the DNA fragments comprising the linker should be extended by 30 bp.

DNase II cleaves sea urchin sperm chromatin giving rise to the 100 bp periodicity and the double-nucleosome repeat simultaneously (fig.2C). Among fragments of 100 bp periodicity those corresponding to double-nucleosome repeat are predominant on the densitogram (fig.2C). The characteristic 2*N*-element of dinucleosomal periodicity consists of a doublet of intensively stained fragments (476 and 384 bp). It seems that both DNA

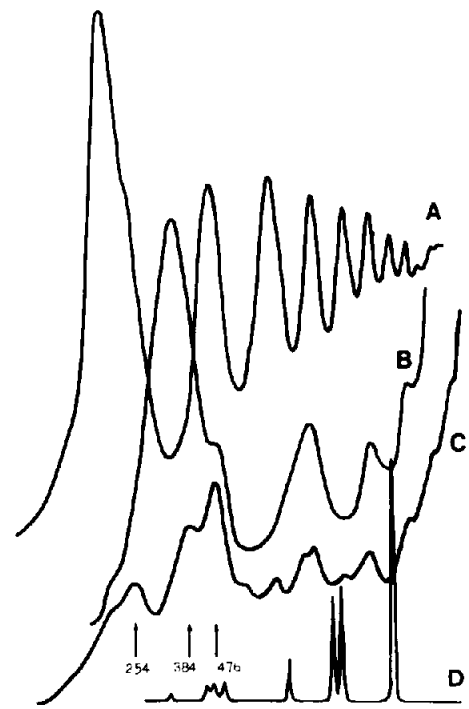

Fig.2. Electrophoresis of DNA fragments isolated from sea urchin sperm nuclei digested with micrococcal nuclease (A), DNase I (B) and DNase II (C). (D) Marker DNA fragments (see fig.1): the size of fragments in bp = 1730, 1162, 804, 489, 454, 432 and 331.

fragments are derived from the nucleodisome at different steps of its cleavage. This conclusion can be drawn also from the fact that the nucleodisome fraction isolated by sucrose density gradient centrifugation are always composed of these 2 DNA fragments (not shown). The band of lower mobility exactly corresponds to the DNA fragment of the undegraded nucleodisome of sperm chromatin (476 bp). The faster DNA band of the doublet, being a product of nucleodisome cleavage, is shorter than the preceeding one by ~100 bp (384 bp). Thus degradation of nucleodisomes in erythrocyte and sperm chromatin, differing in size of linker DNA, is accompanied by cleavage of the 100 bp fragment which appears to be not included in the linker. The linker DNA is likely to be split off as a whole fragment, since the next in size DNA fragment (254 bp), which in erythrocyte chromatin is ~100 bp shorter than the preceeding one, differs in sperm chromatin by 130–140 bp (fig.2C).

Summarizing the data in fig.1,2 one may suggest the main steps of generation of a double-nucleosome repeat.

(1) The dinucleosomal repeat is produced as a result of double-stranded DNA cuts in chromatin at 100 bp intervals. The DNA cleavage sites giving rise to a 100 bp periodicity are located at a distance of 20 bp from both ends of the core DNA as demonstrated in [7].

(2) This pattern of DNA fragmentation is characterized by the relative resistance of linker DNA to DNase II action compared with the intracore sites. The size of fragments including linker DNA varies depending on the nucleosome repeat length.

(3) Intracore sites of every second nucleosome core should be relatively resistant to the nuclease to provide generation of the double-nucleosome repeat. This is true only of the situation in intact nuclei (see below), because analogous experiments failed to reveal clear double-nucleosome repeats after digestion of isolated polynucleosomes [9]. All these considerations are likely to be valid for DNase I also since it cleaves chromatin at 100 bp intervals [9–11] and generates a double-nucleosome repeat [1–3], as does DNase II.

The presumed steps of nucleodisome formation are shown schematically in fig.3. The DNA breaks occur at intracore sites in every second nucleosome, and the resulting nucleodisome is formed as an asymmetric particle. This particle has one extended end including linker DNA and a small part of the core DNA of the next nucleosome. The other end is shortened by the same DNA piece (fig.3, step 2). Complete nucleodisome (like a usual dinucleosome) has a DNA fragment nearly equal in size to dimer DNA (~420 bp in erythrocytes; ~480 bp in sperm chromatin). Then, the intracore 100 bp DNA fragment of the accessible nucleosome in the nucleodisome is split off due to the relative inaccessibility of both intracore sites in every second nucleosome. Later, the linker DNA-containing fragment is cleaved off (fig.3 step 3).

All these experiments with DNase II were performed in the presence of divalent cations. When erythrocyte or sperm nuclei were digested with DNase II in 10 mM Tris–HCl, the ionic conditions

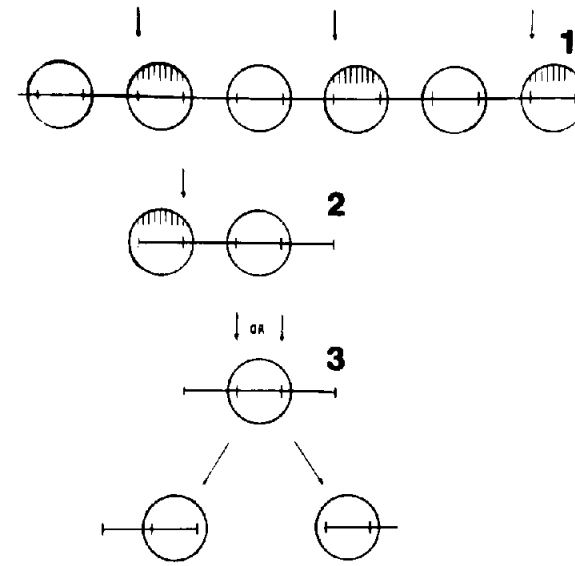

Fig.3. Suggested steps of the formation and subsequent cleavage of nucleodisomes. Each nucleosome core has two sites of cleavage, but the sites appear to be not equivalent in their sensitivity. At the first stage of digestion initial breaks arbitrarily take place at the left site: (1) Chromatin fragmentation at intracore sites in every second nucleosome; (2) Cleavage of the intracore 100 bp DNA fragment from the 'accessible' nucleosome; (3) Cleavage of the 'inaccessible' nucleosome. The arrows mark the sites of preferential DNA cleavage.

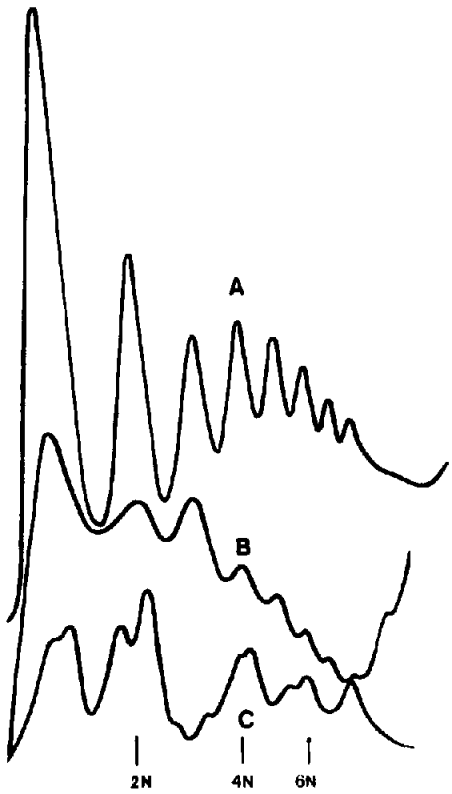

Fig.4. Electrophoresis of DNA fragments isolated from nuclei of sea urchin sperm after treating with DNase II at low ionic strength conditions (10 mM Tris–HCl): (A) micrococcal nuclease digest; (B) DNase II digest at low ionic strength; (C) DNase II digestion in the presence of 3 mM $MgCl_2$.

promoting chromatin unfolding (decondensation), both 100 bp and double-nucleosome periodicity disappear giving rise to the usual DNA nucleosomal repeat at all stages of digestion (fig.4B). This finding implies that the major factor influencing the generation of a double-nucleosome repeat is, in fact, the higher-order chromatin structure.

Our results may be interpreted in terms of supranucleosomal organization of chromatin. Data in favor of nucleosome alternation in compact chromatin appeared in [12]. In spite of the alternation all linkers are accessible to micrococcal nuclease; therefore micrococcal nuclease does not induce the formation of nucleodisomes. At the early stage of digestion DNase II behaves like micrococcal nuclease with respect to cleavage of the linker DNA. Accordingly, the double-nucleosome repeat is not revealed by DNase II at this stage (fig.1B). When DNase II cleaves chromatin at 100 bp intervals a double-nucleosome repeat is generated due to the inaccessibility of the intracore sites in every second nucleosome. The inaccessibility of intracore sites in every second nucleosome is likely to be caused by the alternation of nucleosomes with respect to the location of these sites outside or inside of the solenoid [9]. In such a structure linker DNA is certainly arranged between neighbouring nucleosomes being always accessible to micrococcal nuclease.

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