NUCLEOSOME–NUCLEOSOME INTERACTION IN CHROMATIN

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1. Introduction

The available data suggest that transcriptional activity of chromatin is connected with its structural changes [1,2]. According to the electron microscope evidence, the elementary 100 Å chromatin thread can exist in two main states, i.e., in a compact state, which is characterized by tight packing of nucleosomes and in an extended state when the connecting internucleosomal DNA is clearly seen between the nucleosomes (beads-on-a-string) [3]. It is still unknown which factors are responsible for the structural transition from the closely packed state into the unfolded thread state which is probably indispensable for activation of transcription.

In the presence of divalent metal ions nucleosomes have been shown tightly packed in chromatin [3,4]. Under these conditions internucleosomal DNA interacts with the nucleosome body which provides to it an ordered arrangement in a chromatin thread. In this work we used an enzymatic approach to the study of nucleosome packing in chromatin. Trypsin treatment of chromatin with subsequent nuclease digestion enabled us to characterise in more detail the interactions of internucleosomal DNA with histone complexes of nucleosomes. The data obtained are interpreted to mean that internucleosomal DNA of compact chromatin interacts with the N-terminal parts of nucleosomal histones. Thus the internucleosomal DNA plays a great part in histone–DNA interactions which are important in the close association of nucleosomes. It is supposed that modifications of amino acid residues in N-terminal regions of nucleosomal histones weakens the histone interactions with the internucleosomal DNA, leading to release of internucleosomal DNA and unfolding of the chromatin fiber.

2. Materials and methods

The procedure of nuclei isolation from outbred white-rat thymus and of chromatin fragmentation by nucleases is in [4]. Micrococcal nuclease (Worthington) and the endonuclease from *Serratia marcescens*. (Special Bureau of Biologically Active Substances, Novosibirsk) were used for the experiments. Electrophoresis of DNA fragments under both non-denaturing and denaturing conditions was carried out in polyacrylamide slab gels (13 × 18 × 0.15 cm) [4]. The nuclei were treated with trypsin (Worthington), for 18 h at 4°C (15 μg enzyme/mg nuclear DNA). The reaction was stopped by adding 30 μg trypsin inhibitor/mg nuclear DNA. Histones were extracted with 0.4 M H_2SO_4, precipitated by acetone and subjected to electrophoresis according to [5].

3. Results and discussion

Electrophoretic analysis of DNA fragments produced from chromatin by the two nucleases used revealed clear-cut differences in their action [4]. As compared to the micrococcal nuclease, of which the primary site of action is the internucleosomal DNA, endonuclease from *Serratia marcescens* is able to split both nucleosomal and internucleosomal DNA with about the same efficiency [4,7,8]. Therefore, electrophoresis of DNA fragments from *Serratia* endonuclease chromatin digest performed in non-denaturing condi-

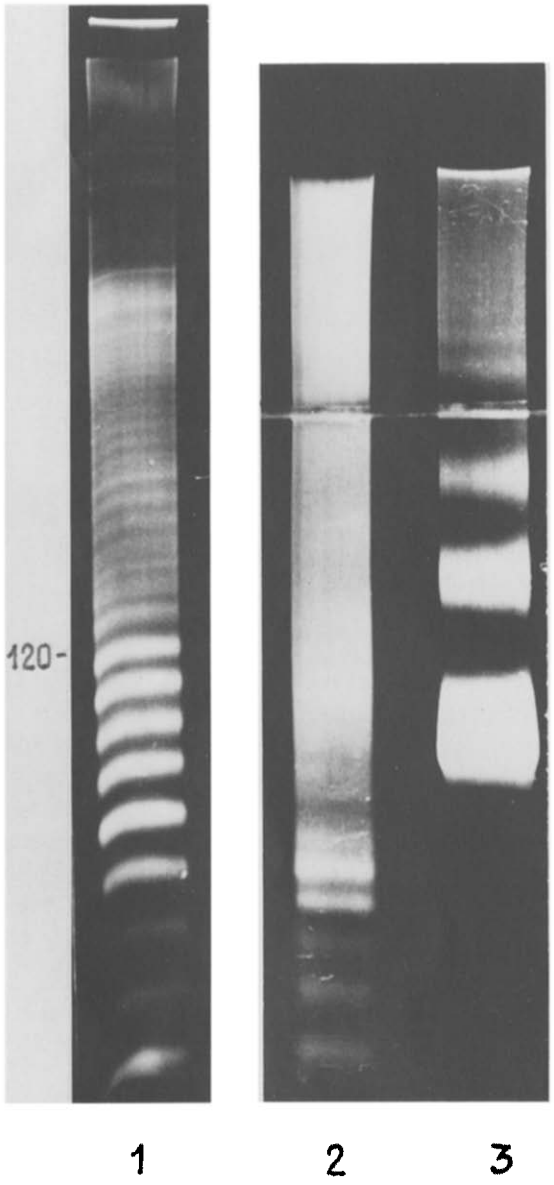


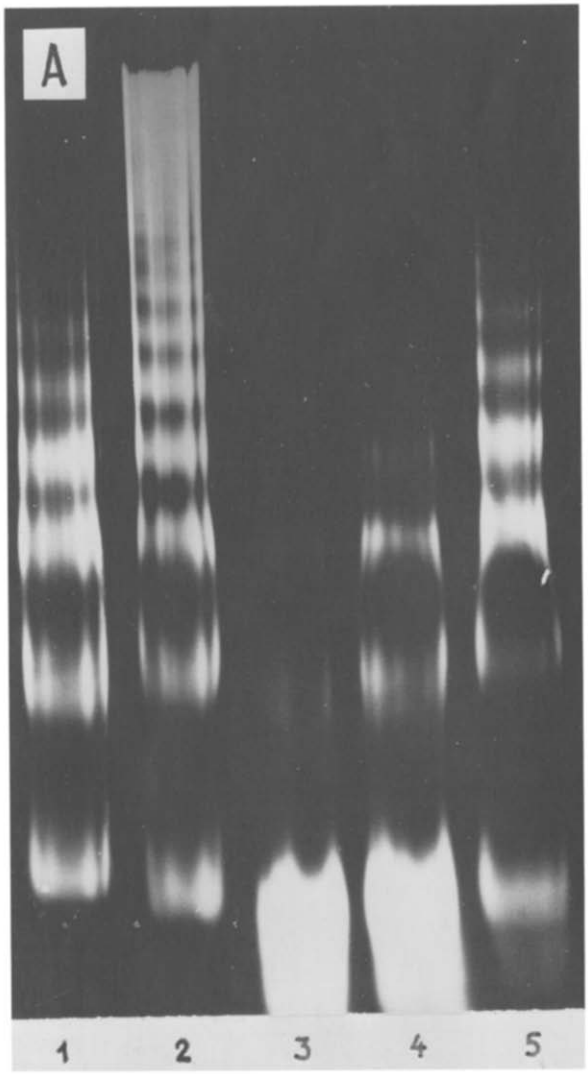
Fig.1. Electrophoresis of the DNA fragments produced by micrococcal nuclease and *Serratia* endonuclease from nuclei. (1) Electrophoresis under denaturing conditions: Serratia endonuclease (100 units, 2 min). (2, 3) Electrophoresis under non-denaturing conditions: (2) *Serratia* endonuclease digest of nuclei (30 units, 15 min); (3) micrococcal nuclease digest of nuclei (30 units, 15 min). Deproteinized DNA was denatured by heating in the electrophoretic buffer containing 6 M urea.

tions does not shown the large-scale periodicity characteristic of micrococcal nuclease chromatin digest. On the other hand, under denaturing conditions of electrophoresis a series of single-stranded fragments of $\geqslant 300$ nucleotides, which are multiples of 10 nucleotides, is found in *Serratia* endonuclease chromatin digest (fig.1). In this respect, the effect of *Serratia* endonuclease is similar to that of pancreatic DNase I [6,9]. The presence of a large ladder of single-stranded fragments, which are multiples of 10 nucleotides and are > 200 nucleotides, indicates that both internucleosomal and nucleosomal DNA are protected by histones from nuclease attack [7,8].

The long-range 10-nucleotide periodicity of nuclear DNA fragmentation can be detected in various conditions of nuclease digestion: at 0.1–5 mM Mg^{2+}, in the presence of 1 mM $CaCl_2$ or 0.15 M NaCl. Consequently, the compact packing of internucleosomal DNA is rather stable in these ionic conditions.

We had also shown that the 10-nucleotide periodicity in fragmentation of internucleosomal DNA is retained after removal of H1 histone [7,8]. The procedure used for removal of H1 histone did not cause clustering of nucleosomes. DNA repeat length characteristic for micrococcal nuclease digest of nuclei does not change in H1-depleted chromatin (fig.2). Consequently, the ordered packing of internucleosomal DNA in chromatin is likely to be caused not by histone H1 but by the interaction of internucleosomal DNA with the other histones. These histones form in the nucleosome body an octamer, in which a hydrophobic globular core and extended, charged N-terminal parts are distinguished [10]. When chromatin is treated with trypsin, about 20–30 amino acid residues are split off from the N-terminal parts of histones, along with digestion of non-histone proteins and H1 histone [11,12]. The hydrophobic non-hydrolyzed core of histones remains to bind to DNA and is able to protect a small stretch of DNA from nuclease digestion. We attempted to get more detailed information on the interaction of internucleosomal DNA with the histone octamer by treating chromatin with trypsin before nuclease digestion.

The results of the electrophoretic analysis of histones isolated after trypsin treatment of the nuclei (fig.3) are in good agreement with the published data obtained after trypsination of chromatin or nucleosomes [11–13]. Trypsination causes lysis of the



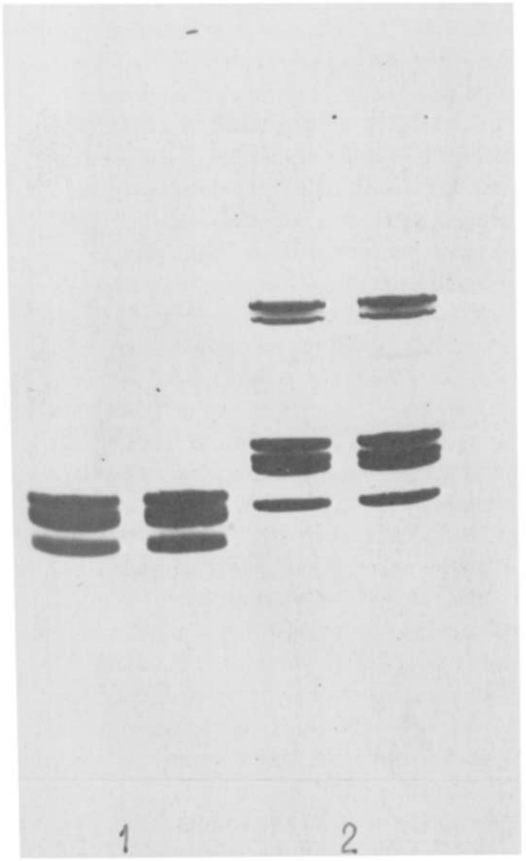


Fig.3. Electrophoresis of histones isolated from trypsinized nuclei. Histones were dissolved in an electrophoretic buffer (0.05 M Tris–glycine buffer, 0.1% SDS, 1% mercaptoethanol), heated for 5 min and separated in 15% polyacrylamide gel [5]: (1) histones from trypsinized nuclei; (2) control.

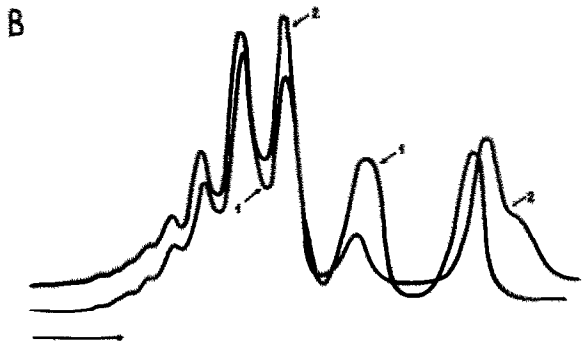


Fig.2. Agarose gel electrophoresis of DNA fragments produced by micrococcal nuclease in H1-containing and H1-depleted chromatin. (A) Electrophoresis in 1% agarose: (1) DNA fragments from H1-containing oligonucleosomes, 2 min digestion; (2) DNA fragments from nuclei (control); (3–5) DNA fragments from H1-depleted oligonucleosomes. (3) 10 min digestion; (4) 5 min digestion; (5) 2 min digestion. (B) Densitometric scans of runs 1 and 5 from A: (1) H1-containing oligonucleosomes; (2) H1-depleted oligonucleosomes. Nuclei were digested by micrococcal nuclease (30 units, 10 min), centrifuged and suspended in 0.6 M NaCl [7]. After chromatography on Sepharose 4B column the excluded peak was collected, dialyzed and repeatedly digested with 10 units micrococcol nuclease.

nuclei which results in formation of a viscous chromatin solution. The action of trypsin was stopped by addition of trypsin inhibitor and after that the chromatin was digested by *Serratia* endonuclease. The degradation rate of trypsinized chromatin increased drastically (fig.4). Electrophoresis of the resulting DNA fragments both under non-denaturing and denaturing conditions revealed also considerable changes in the pattern of DNA fragmentation.

When analyzing the electrophoretic pattern of DNA fragments from the control chromatin under non-denaturing conditions we observed no discrete fragments corresponding to the DNA of mononucleosomes, their dimers, trimers and so on (fig.1). On the contrary, after nuclease digestion of trypsinized chromatin with *Serratia* endonuclease a clear large-scale periodicity of fragments was detected (fig.5).

Thus, it appears that the splitting of the N-terminal parts of histones (and H1 histone) changes accessibility of chromatin to *Serratia* endonuclease: the internucleosomal DNA of trypsinized chromatin becomes the most sensitive site to endonuclease attack. This effect is particularly strongly revealed in electrophoresis under denaturing conditions. As compared to the control chromatin digest with a continuous spectrum of DNA fragments up to 300 and more nucleotides, which are multiples of 10 nucleotides (see fig.1), the pattern of DNA fragmentation of trypsinized chromatin disclose an additional periodicity, which is equivalent to that of micrococcal digest of intact chromatin (fig.6). The 10-nucleotide periodicity is also retained, but it may be followed only to $\simeq$ 140 nucleotides [12,13]. No 10-nucleotide periodicity was found in the region between the position of mononucleosomal and dinucleosomal DNA.

It has been shown that the regular 10-nucleotide periodicity of DNA fragmentation up to 250 nucleotides and more persists after removal of histone H1 [7,8]. It is disturbed, however, after trypsin splitting of the N-terminal parts of nucleosomal histones H2A, H2B, H3 and H4. This is caused by a rapid and specific degradation of internucleosomal DNA in trypsinized chromatin. In intact chromatin, this DNA stretch is cleaved by *Serratia* endonuclease, along with nucleosomal DNA, into fragments, which are multiples of 10 nucleotides. However, after loss of the N-terminal parts of the histone the internucleosomal DNA becomes much more accessible to this endonuclease

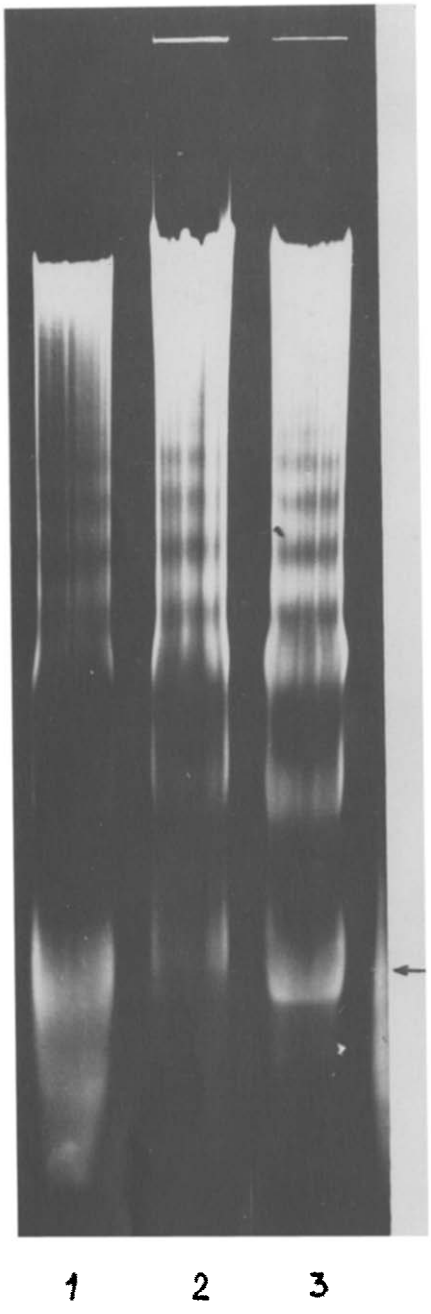


Fig.4. Kinetics of degradation of trypsinized chromatin by *Serratia* endonuclease: (1) control chromatin (1000 units); (2) trypsinized chromatin (500 units).

as compared to nucleosomal DNA.

These data permit us to suggest that in the compact state of chromatin, with tightly packed nucleosomes, internucleosomal DNA interacts with the N-terminal parts of nucleosomal histones. Such interaction is likely to induce coiling of internucleosomal DNA similar to that of DNA in nucleosomes [3], and it also provides for close contact between nucleosomes. Modification of the nucleosomal histones (e.g., acetylation, methylation) [14], which occurs primarily in N-terminal parts of molecules may weaken or even disturb completely the interaction of internucleosomal DNA with histones. This may lead to weakening of the close contacts between nucleosomes and unfolding of the chromatin fiber, i.e., induce the transition of the chromatin thread into a 'beads-on-a-string' state. It may be supposed that such transition of the chromatin thread is indispensable for transcription.

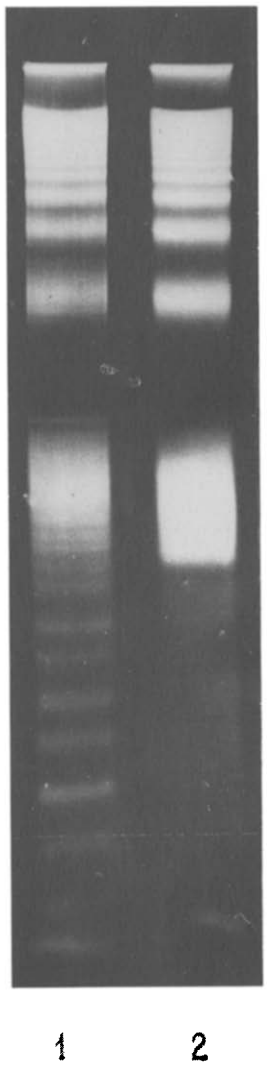


Fig.5. Electrophoresis under non-denaturing conditions of the DNA fragments from trypsinized chromatin. DNA fragments from *Serratia* endonuclease digest of trypsinized chromatin: (1) 500 units, 2 min (30% acid-soluble products); (2) 50 units, 2 min (13% acid-soluble products); (3) DNA fragments of micrococcal nuclease chromatin digest (control). The arrow indicates the position of mononucleosomal DNA.

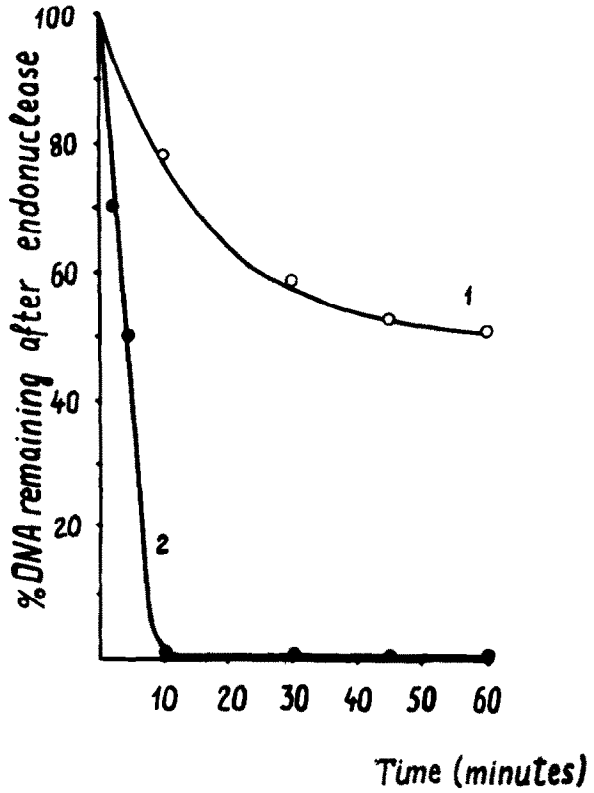


Fig.6. Electrophoresis under denaturing conditions of the DNA fragments from trypsinized chromatin: (1) DNA fragments from *Serratia* endonuclease digest of trypsinized chromatin (50 units, 2 min); (2) DNA fragments from micrococcal nuclease chromatin digest (control).

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