Temperature-dependent cleavage of chromatin by micrococcal nuclease near the nucleosome center

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Digestion of nuclei at 4°C with micrococcal nuclease results in significant intranucleosomal cleavage compared to digestion conducted at 37°C. Employing nucleoprotein gel electrophoresis in one dimension followed by DNA electrophoresis in a second dimension, we demonstrate that such temperature-sensitive, internal cleavage predominantly occurs about 20 bp from the nucleosome center. We suggest that lower temperatures reduce the stability of hydrophobic interactions within the histone octamer and lead to a conformational alteration in nucleosomes that is detected by micrococcal nuclease.

Chromatin structure *Nucleosome* *Half-nucleosome* *Histone octamer* *Micrococcal nuclease* *Two-dimensional electrophoresis*

1. INTRODUCTION

Micrococcal nuclease has been widely used as an enzyme in chromatin research because of its marked specificity to cleave linker regions between nucleosome cores. Previous investigators have noted that when nuclei are digested with this enzyme near 0°C, trimming of linker regions due to exonucleolytic activity is nearly eliminated [1,2], but increased cleavage occurs within nucleosomes [2]. We report here that the temperature-sensitive, internal cleavage of nucleosomal DNA by micrococcal nuclease occurs predominantly within a specific region of the nucleosome core.

2. MATERIALS AND METHODS

Nuclei were isolated from MPC-11 plasmacytoma cells as described in [3]. After washing by repeated low-speed centrifugation at 4°C in digestion buffer (4 mM $MgCl_2$, 1 mM $CaCl_2$, 5 mM sodium butyrate, 1 mM iodoacetamide, 1% thiodiglycol, 1 mM phenylmethylsulfonyl fluoride, 300 mM sucrose, 50 mM triethanolamine, 25 mM KCl, pH 7.4, at 37°C), nuclei were suspended to 8 mg/ml (as DNA) and digested either at 4°C with 400 units micrococcal nuclease (Worthington) per mg DNA for 30 min, or at 37°C with 16 units micrococcal nuclease per mg DNA for 10 min (after 5 min preincubation). After centrifugation, the S2 chromatin fraction was prepared by lysis of the digested nuclear pellet with 2 mM EDTA, pH 7.4 [3,4]. Gel electrophoreses of nucleosome components were performed as in [5], with the following minor modifications. Nucleoprotein slab gels 0.3 cm thick and 20 cm long contained 3.5% polyacrylamide, 0.5% agarose and 30% glycerol. Wells 0.8 cm wide, loaded with 20 μg samples (as DNA) in 50% glycerol, 1 mM EDTA (pH 7.4), were used to cut sample strips 0.5 cm wide for loading across the top of second-dimension DNA gels. Non-denaturing DNA gels consisted of 5% polyacrylamide, 0.1% SDS. When required, gels were fixed in 50% methanol to remove SDS and

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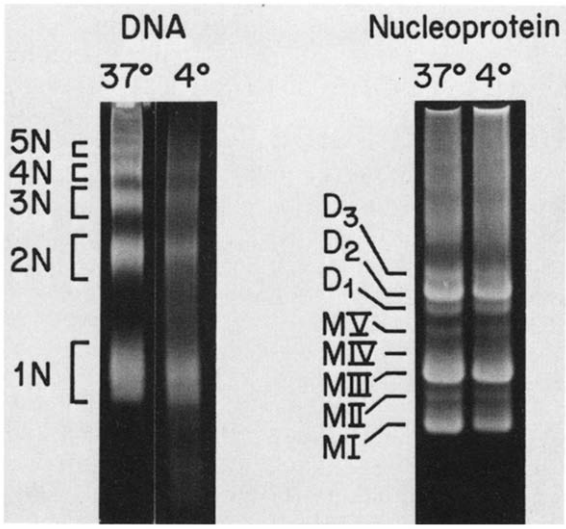
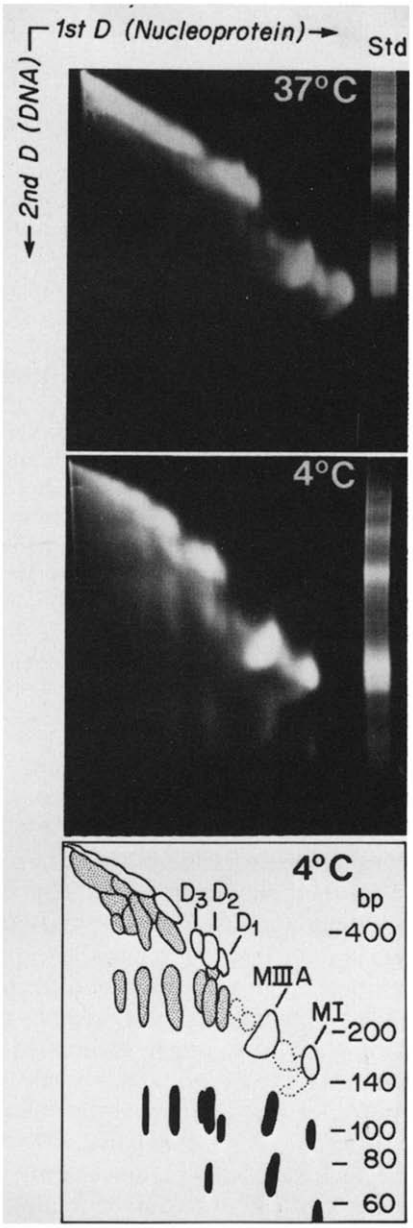


Fig.1. Electrophoretic profiles of purified DNA and nucleoprotein particles. Samples were prepared from nuclei digested at either 37 or 4°C with micrococcal nuclease (as indicated). Mono- and oligonucleosomal fragments (1N–5N) and different electrophoretic forms of mononucleosomes (MI–MV) and dinucleosomes (D_1–D_3) are shown. (See [5] for nomenclature and further details regarding different electrophoretic forms of nucleosomes.)

stained with 1 μg/ml ethidium bromide dissolved in electrophoresis buffer.

3. RESULTS AND DISCUSSION

In agreement with [2], electrophoresis of DNA derived from nuclei digested at either 37 or 4°C with micrococcal nuclease reveals a higher internucleosomal band background for the 4°C sample (fig.1) (see below). In contrast, nucleoprotein gel electrophoresis of soluble chromatin samples prepared from such nuclei reveals nearly identical patterns, suggesting that the nucleosomal particles of lower temperature digests, which contain internally cleaved DNA, remain largely intact (fig.1)



DNA standard (Std) of the sample is on the right of second-dimension gels. The diagram depicts as open spots major electrophoretic forms of intact mononucleosomes (MI and MIIIA), dinucleosomes (D_1–D_3), and oligonucleosomes. Products of internal cleavage below mononucleosomal DNA lengths are indicated as closed spots, while suboligomeric products of internal cleavage greater than mononucleosomal DNA length are indicated as stippled spots. Minor mononucleosomes MII, MIV and MV and other components are indicated as dashed open spots.

Fig.2. Two-dimensional electrophoresis of nucleosome components. Chromatin samples prepared from nuclei digested at either 37 or 4°C with micrococcal nuclease were separated by nucleoprotein electrophoresis in the first dimension, and by non-denaturing DNA electrophoresis in the second dimension (as indicated). A

(see below). Therefore, we decided to map the position(s) of temperature-sensitive, intranucleosomal cleavage by performing nucleoprotein gel electrophoresis in one dimension, followed by DNA electrophoresis in non-denaturing gels in a second dimension.

The two-dimensional gel patterns shown in fig.2 confirm the presence of temperature-sensitive, internal cleavages of nucleosomal DNA, since the sample prepared from the 37°C digest possesses little background below the diagonal, whereas the 4°C digest exhibits a series of characteristic smaller fragments. The DNA lengths of these fragments were determined by calibrating gels with *Hae*III-cleaved ϕX174 (not shown). It is noteworthy that two fragment size classes occur below mononucleosomal bands and that the sum of the lengths of these components equals the DNA lengths of the intact parent particles (fig.2). Intact mononucleosomes lacking histone H1 (MI) have a DNA length range of 146–184 bp and give rise to fragments of 87–106 and 47–62 bp. Intact mononucleosomes containing histone H1 (MIIIA) have a DNA length range of 166–220 bp and give rise to fragments of 97–131 and 63–86 bp. From this information and the 1.75-turn, 146 bp nucleosome core model [6,7], it can be deduced that micrococcal nuclease cleavage occurs internally about 20 bp from the nucleosome center at 4°C. Oligonucleosomes are also internally cleaved at an analogous position, since dinucleosomes, for example, give rise to the same submononucleosomal DNA fragments noted above, besides yielding fragments intermediate in size between mono- and dinucleosome in length (fig.2).

It should also be noted that the temperature-dependent effect is reversible. Preincubation of nuclei at 37°C for extended periods does not eliminate the capacity for subsequent internal cleavage at 4°C. The position of the temperature-sensitive cleavage mapped here corresponds to a major site of primary and secondary attack by micrococcal nuclease on isolated, end-labeled core particles, as identified by an analysis of cleavage products on non-denaturing gels [8]. It is of further interest that this site is symmetrical and corresponds to regions which are believed to be in close contact with histones H3 and H4 [9,10]. Thus, it is possible that the reduced temperature weakens hydrophobic interactions [11] within the H3-H4 tetramer and/or other regions of the histone octamer. Such perturbations may lead to the observed heightened sensitivity of this region to micrococcal nuclease. Less likely alternatives are temperature-dependent changes in DNA helical twist [12] or micrococcal nuclease cleavage specificity per se.

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