DIGESTION OF CHROMATIN TO H1-DEPLETED 166 BASEPAIR PARTICLES BY Ca^{2+}/Mg^{2+}-DEPENDENT ENDONUCLEASE

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1. Introduction

Digestion of chromatin with micrococcal nuclease led to discern between a nuclease-sensitive linker region and a more refractory core particle containing 146 basepairs (bp) of DNA [1]. Later studies revealed a further barrier to digestion at ~160 bp [2]. Particles of 160 bp prepared in [3] contained histone H1, but core particles lack this histone [4]. On the other hand, 168 bp fragments were generated during digestion of nuclei depleted of H1 by acidic treatment [5]. A disadvantage of using micrococcal nuclease is its exo- and endonucleolytic activity, although it is possible to change the ratio of both activities by varying the digestion conditions [4,5]. Here, we employed the Ca^{2+}/Mg^{2+}-dependent endonuclease from rat liver, which lacks an exonucleolytic activity [6,7]. We observed that 166 bp particles depleted of H1 are highly refractory to this endonuclease.

2. Materials and methods

Rat liver nuclei were prepared as in [8] and digested with endogenous endonuclease at 37°C in buffer 'O' [9], containing 0.5 mM phenylmethanesulfonyl fluoride. The reaction was terminated by adding EGTA to 2.7 mM. Nuclei digested for 30 min were also dialysed and sedimented in isokinetic sucrose gradients as in [8] but for 3 h and in 13.3 mM KCl, 6.7 mM NaCl, 0.4 mM $MgCl_2$, 15 mM Tris-acetate (pH 8.0), 0.1 mM EGTA. Secondary digestion by S1-nuclease was performed at 22°C as in [8].

Ca^{2+}/Mg^{2+}-dependent endonuclease was purified as in [8] followed by a precipitation with ammonium sulfate between 50–85% saturation [10]. Enzyme activity was defined as 1 unit equal to the amount of enzyme that catalyzes 1 doublestrand break/λ DNA in 1 h under standard assay conditions (in preparation). H1-Depleted nucleosomal chains (20–30 nucleosomes long) were prepared from nuclei mildly predigested with micrococcal nuclease, using a modification (in preparation) of the procedure in [11]. They were then digested with Ca^{2+}/Mg^{2+}-endonuclease (~7.4×10^4 units/ml) at 37°C in 5 mM Tris–HCl (pH 7.5), 25 mM KCl, 0.9 mM $CaCl_2$, 0.9 mM $MgCl_2$). DNA and histones were extracted as in [8,12]. DNA was electrophoresed on 6% polyacrylamide slab gels [8] and histones on a 15% polyacrylamide slab gel in the presence of sodium dodecyl sulfate [13]. The length of the monomeric DNA fragments was estimated by tracing the negatives with a Beckman DU-8 spectrophotometer and the use of φX 174 RF DNA–*Hae*III restriction fragments (BRL).

3. Results

Rat liver nuclei were digested with endogenous endonuclease for various times, and the purified DNA was electrophoresed on a 6% polyacrylamide gel. As shown by the ethidium bromide staining in fig.1, monomeric fragments appearing after 4–8 min incubation were rapidly decreased in size to reach a plateau at 166 bp. The barrier of digestion at 166 bp was apparent from 12–120 min incubation (fig.4). In nuclei containing mostly mononucleosomes already at zero time of incubation due to digestion during their preparation, 166 bp fragments represented the most prominent component for 120 min (not shown). The predominant band at 166 bp persisted during secondary digestion of the DNA with S1 nuclease

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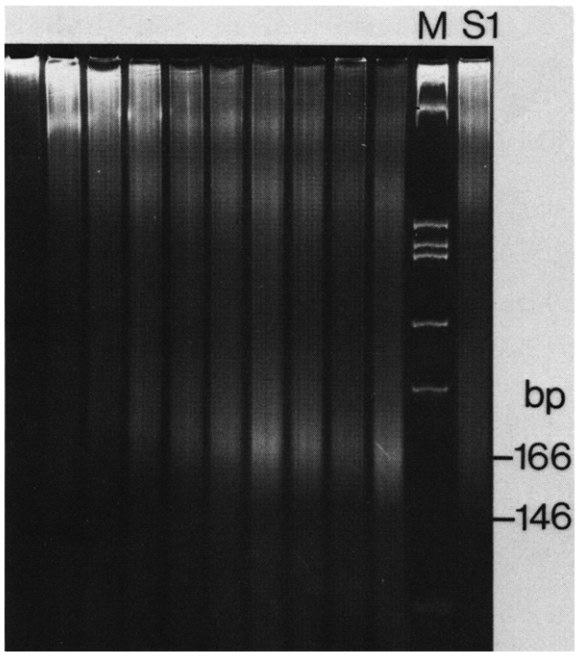

Fig.1. Time course of digestion of rat liver nuclei with endogenous endonuclease. Digestion time, from left to right, was 0, 4, 8, 12, 16, 20, 30, 40, 60 and 90 min; M, marker fragments; S1, DNA from a 12 min incubation subjected to a secondary digestion with S1 nuclease.

(fig.1). These results indicated that 166 bp particles are highly refractory to digestion with Ca^{2+}/Mg^{2+}-dependent endonuclease.

To analyze the DNA and histone composition specifically in mononucleosomes, soluble chromatin from nuclei digested for 30 min was prepared and sedimented in a buffer containing 20 mM monovalent cations and 0.4 mM Mg^{2+}. The material on the gradient (fig.2A) was recovered as a fraction (1) containing mostly mononucleosomes, a fraction (2) containing oligonucleosomal chains and composite particles [12], and finally a fraction (3) including the supranucleosomal particles observed as metastable entities during endonuclease digestion [8]. The DNA gel in fig.2B shows that monomers contained DNA fragments of (on the average) 166 bp long, while the monomeric fragments in fractions 2 and 3 exhibited sizes ~10–20 bp longer. The histone gel in fig.2C shows that the mononucleosomal fraction contained amounts of H1 (related to the amount of core histones) equivalent to <5% of the amount found in intact nuclei. The oligonucleosomal fraction was slightly depleted of H1, while frac-

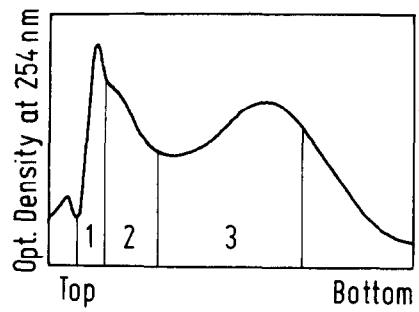

Fig.2A.

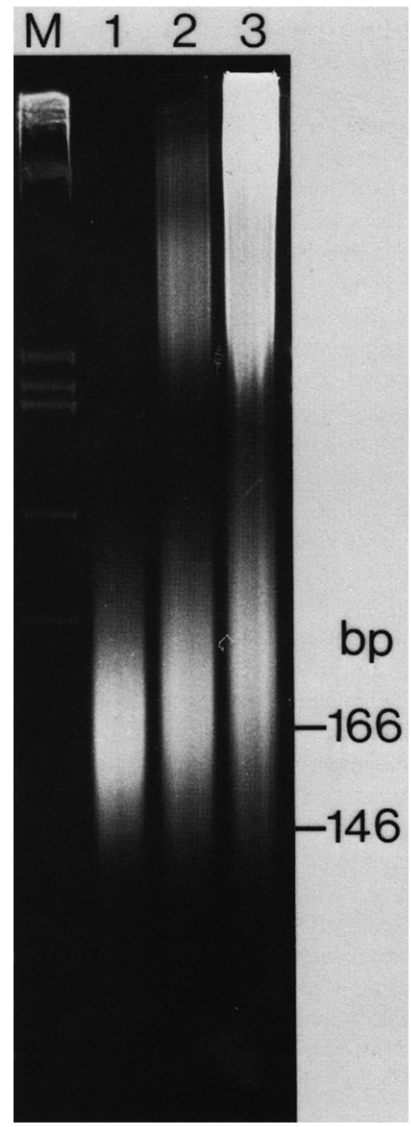

Fig.2B.

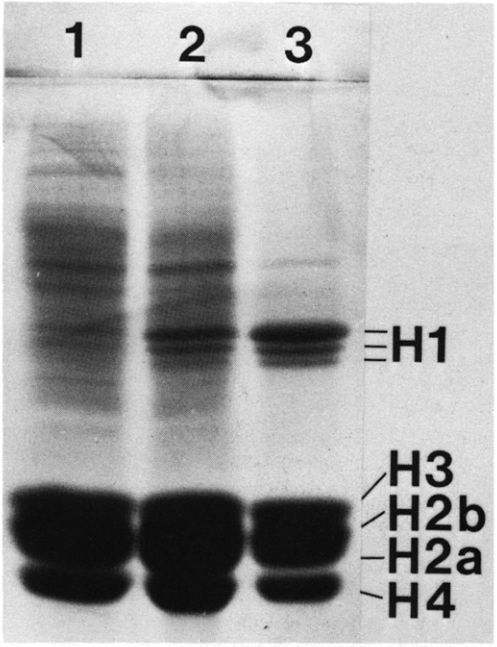

Fig.2C.

Fig.2. DNA and histone composition in soluble chromatin fractions separated by sucrose gradient sedimentation: (A) Sedimentation profile – fractions recovered from the gradient are designated as 1, 2 and 3; (B) electrophoresis of the purified DNA on a 6% polyacrylamide gel; (C) electrophoresis of acid extracted histones on a 15% polyacrylamide gel.

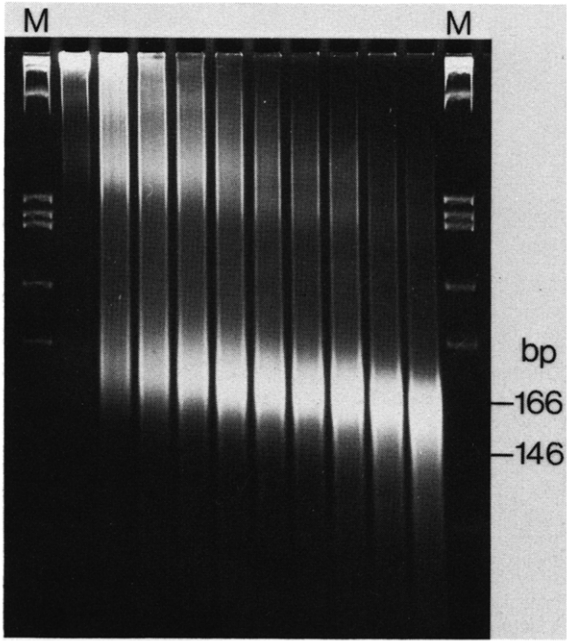

Fig.3. Digestion of H1-depleted chromatin with added Ca^{2+}/Mg^{2+}-dependent endonuclease for 0, 5, 10, 15, 25, 35, 45, 55, 85 and 115 min (left to right).

tion 3 contained H1 at 1.0–1.1-times the amount of H1 in intact nuclei. These results indicate that the mononucleosomal fraction is enriched for 166 bp particles and that these are depleted of H1. The small amount of H1 in the mononucleosomal fraction may result from a contamination of dinucleosomes or from mononucleosomes with DNA >166 bp.

The most simple explanation of the above results is that H1 redistributes as soon as mononucleosomes are trimmed down to 166 bp. This may allow the conclusion that H1 is not required to protect 166 bp particles from further degradation to 146 bp cores. However, this conclusion only holds if the possibility can be excluded that H1 migrated from mononucleosomes at preparative steps following the digestion period. To this end, long-chained H1-depleted chromatin was prepared by a gentle ion-exchange method [11] and digested with added endonuclease for various times. The gel in fig.3 shows that the linker DNA was rapidly degraded within the first 5 min incubation producing particles containing 165–175 bp of DNA. Continuing incubation further cleavage was drastically reduced (fig.4), although enzyme activity was maintained up to the end of the incubation period. This shows that endonuclease-refractory particles with ~166 bp DNA are also produced from H1-depleted chromatin.

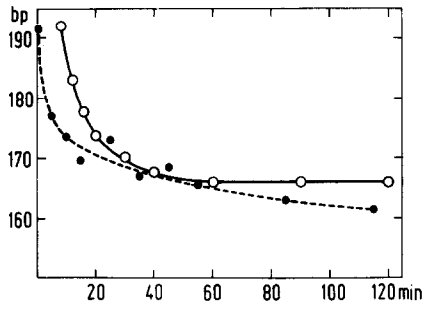

Fig.4. Size of the monomeric DNA fragments. The negatives of the gels in fig.1,3 were scanned and the size of the fragments at maximal intensity were plotted as a function of the incubation time: (o——o) nuclei; (•– – –•) H1-depleted chromatin.

4. Discussion

This study shows that mononucleosomes containing ~166 bp of continuous DNA but lacking H1 are highly refractory to digestion with Ca^{2+}/Mg^{2+}-dependent endonuclease. As digestion was performed in a quasi-physiological ionic milieu [9], endonuclease-refractory 166 bp particles lacking H1 appear to represent a feature of native chromatin structure. We concluded that H1 is not required to protect the DNA ends in 166 bp particles from a nicking activity of this endonuclease or from being shortened by its doublestrand cleavage activity. Instead, the core histones appear to have enough contact to the DNA ends to drastically slow down further digestion. Consistent with this, strong crosslinking of H3 was found in undigested chromatin to 165 bp DNA over a stretch close to the ends [14]. This study also describes a method to prepare 166 bp particles by the use of Ca^{2+}/Mg^{2+}-dependent endonuclease and to separate these from H1-containing particles with larger DNA sizes by sedimentation in a physiological ionic milieu. Our results with Ca^{2+}/Mg^{2+}-dependent endonuclease should be contrasted to the action of micrococcal nuclease which produces 166 bp particles of a relatively short half-life and stable 146 bp particles [1]. This supports the notion that core particles are generated predominantly by the exonucleolytic activity of micrococcal nuclease [4,5].

To discuss the location of H1, it seems to be important to emphasize that the core histones by themselves can wind 166 bp of DNA into 2 complete turns. The central part of H1 crosslinks with H2A [15] which is located on the side of the nucleosomes [16]. In an attempt to explain this observation in combination with our results, we entertain the hypothesis that the central part of H1 occupies a position on one side of the nucleosome. Consistent with this, H1 was found to be located asymmetrically on the nucleosome [17] and in a position where the DNA enters and leaves the nucleosome [11]. The proposed location would also explain the crosslinking of H1 along the whole length of the nucleosomal DNA [14] and the observation of 2 binding sites for lysine-rich histones [18,19].

Acknowledgements

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