STRUCTURAL ORGANIZATION OF CALF THYMUS CHROMATIN DEPLETED OF HISTONE H1 BY ACIDIC TREATMENT

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1. Introduction

Several methods have been described to selectively extract histone H1 from nucleohistone [1–3]. The study of these types of chromatin may help to understand the structural role played by histone H1. In [3] selective extraction by acid was used. This method does not disturb the sequential arrangement of nucleosomes on DNA, as do other methods [4–6]. However, DNA is denatured by the acidic treatment. We have found that this denaturation is reversible, so that when the pH of the H1-depleted chromatin is adjusted to a neutral value, the DNA returns to its native state and the nucleosomal organization is recovered.

This type of H1-depleted chromatin offers an interesting material to study the effect of nuclease digestion. We have found that the enzyme degrades this type of chromatin much faster than native chromatin but, surprisingly, the degradation of the nucleosome particle (trimming) to give core particles with ~145 basepairs, proceeds at a slower rate, so that nucleosomes with ~160 basepairs are obtained in spite of the absence of histone H1. These observations indicate the importance of the conformation of DNA in understanding the effect of nucleases on the nucleosomes.

2. Experimental methods and results

Purified calf thymus nuclei were obtained by homogenization of the gland in 0.3 M sucrose, 1 mM $CaCl_2$, 5 mM $MgCl_2$, 25 mM KCl, 10 mM Tris–HCl (pH 7.7). The nuclei were depleted of histone H1 by the method in [3]. The nuclei were extracted with a pH 2.2 buffer and selective extraction of histone H1 was thus achieved (fig.1). This pH-value is rather critical, since at pH 20, other histones were extracted with H1, and pH 2.4, some histone H1 remained in the acid-treated chromatin. After acid extraction the nuclei were recovered by centrifugation and were washed twice in the above buffer.

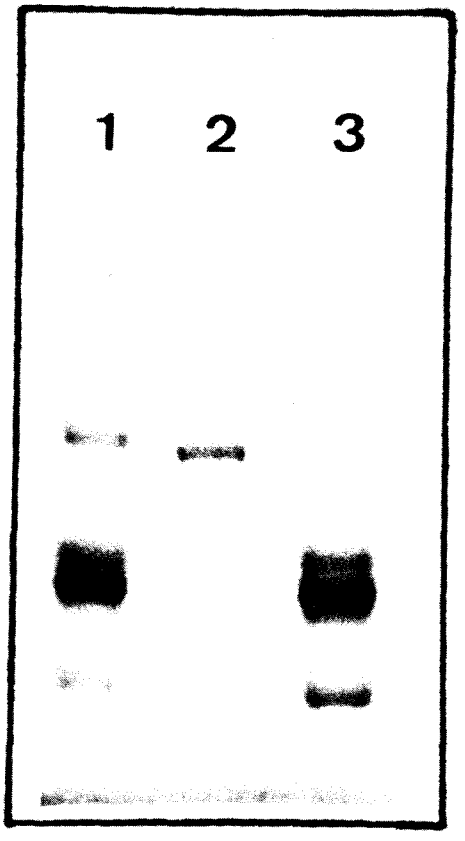

Fig.1. Gel electrophoresis of histones from intact calf thymus nuclei (1) and after extracting histone H1 by treatment at pH 2.2 (3). The protein extracted is shown in (2).

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The nucleohistone thus prepared contained the full complement of histones, except H1. The histone/DNA ratio was 0.88 as determined by the method in [7]. Thermal denaturation of this chromatin showed a bimodal transition as found in native calf thymus chromatin [8]. The main difference was that the first melting transition occurred at 65.5°C instead of 69.8°C, but the overall shape of the curve was the same in both cases. In particular, the total hyperchromicity of the sample was found to be 43%, indicating that no appreciable amount of denatured DNA was present in the starting material.

The state of this chromatin was also ascertained by X-ray diffraction from fibers, using the procedures in [9]. The results obtained are shown in fig.2. The humidity conditions were chosen to obtain a weight concentration of 50–60% in each case, as ascertained by weighing the wet and dehydrated samples. The fibers studied at pH 2.2 only show a ring at ~5 nm and a broad ring at ~1 nm. The latter ring is probably due to the protein, perhaps with a contribution of denatured DNA. The intensity distribution of this ring is quite different from that of native nucleohistone, which has a maximum at ~1.2 nm and a shoulder at ~0.8 nm, as shown in [10] and fig.2A.

The ring observed at ~5 nm in the acid-treated nucleohistone can not be interpreted unambiguously. Probably it corresponds to the first subsidiary peak of nucleosome-like particles made of histone cores sur-

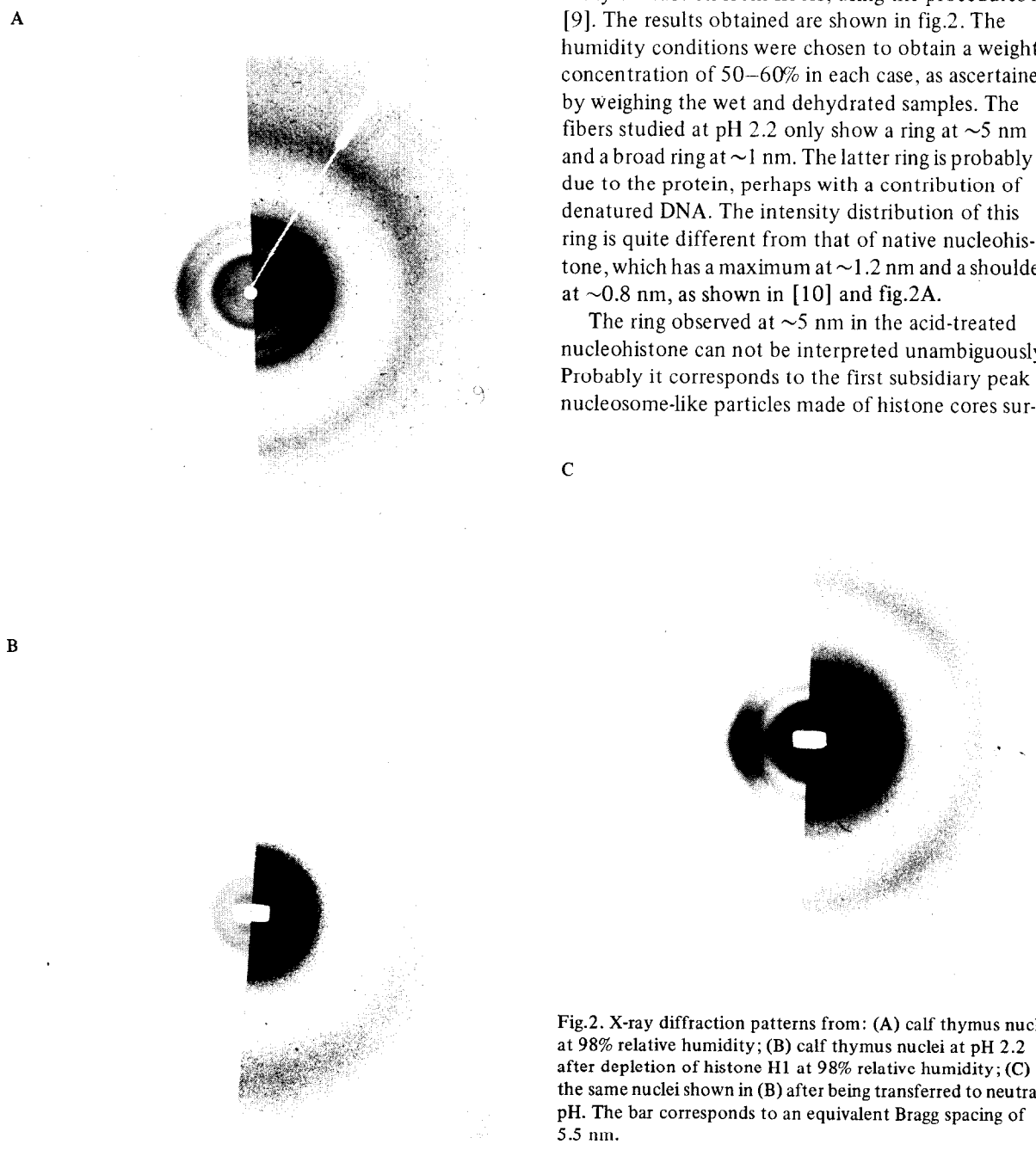

Fig.2. X-ray diffraction patterns from: (A) calf thymus nuclei at 98% relative humidity; (B) calf thymus nuclei at pH 2.2 after depletion of histone H1 at 98% relative humidity; (C) the same nuclei shown in (B) after being transferred to neutral pH. The bar corresponds to an equivalent Bragg spacing of 5.5 nm.

Table 1
Number of basepairs in the DNA of oligonucleosomes after nuclease digestion

Sample	Digestion time (min)	% DNA soluble in perchloric acid	Number of basepairs			
			Monomer	Dimer	Trimer	Tetramer
Calf thymus nuclei	2	6.7	–	343	549	772
	4	9.9	168	335	525	733
	7	13.2	158	326	502	708
	10	15.0	152	320	495	676
	120	36.7	144	302	–	–
H1-depleted calf thymus nuclei	1	5.3	168	339	531	708
	4	11.2	162	331	519	–
	10	23.1	165	324	510	–
	20	39.5	157	–	–	–

rounded by denatured DNA. An approximately spherical particle of this type should in fact give a peak in this region [11].

When the acid-treated nucleohistone is transferred to neutral pH, the X-ray pattern is indistinguishable from that of native chromatin (fig.2C). Under these conditions the DNA has recovered its native organization and the nucleosomes have their characteristic spatial arrangement.

Micrococcal nuclease digestion of nuclei was done at 37°C with 50 Boehringer units of enzyme/mg DNA in the above buffer. Digestion was stopped by addition of EDTA and chilling in ice. The DNA in the digest was extracted as in [12] and was characterized in slab gels, according to [13]. The gels were calibrated with *Hae*III restriction fragments from PM2 DNA. The size of the DNA bands was determined from the position of the peaks in microdensitometer tracing. The monomer band was slightly asymmetric, particularly after long digestion times, indicating the presence within this band of some DNA with smaller dimensions (see table 1).

In fig.3 we show the amount of monomer DNA recovered as a function of time. It is clear that the chromatin is cleaved much faster when histone H1 has been removed. However, the size of monomer DNA does not diminish very fast in H1-depleted chromatin (fig.4, table 1). This is a striking result, since one would expect a much faster trimming of nucleosomal DNA in the absence of histone H1. It appears that the 160 basepair nucleosomal particle obtained from this type of chromatin is more stable towards nuclease digestion. The significance of the results in fig.4 is reinforced if it is taken into account that the relative amount of monomer DNA present in the preparations is much higher in the H1-depleted nuclei

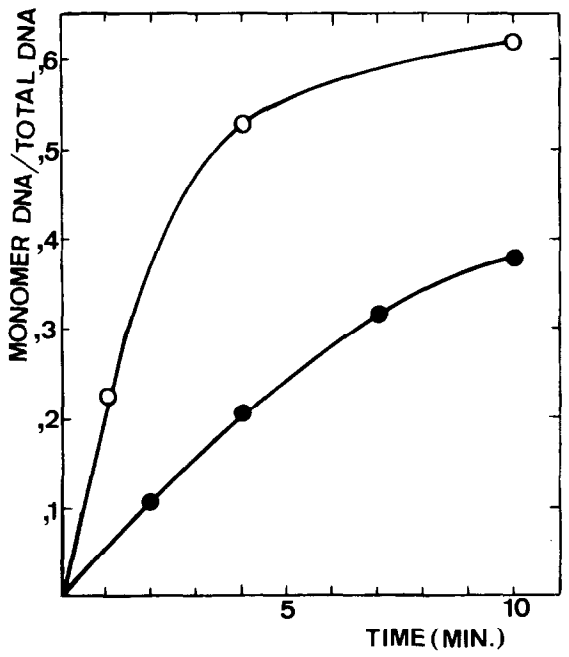

Fig.3. Relative amount of monomer DNA *vs* total DNA recovered in the digest as a function of time. The values plotted correspond to both whole nuclei (•) and H1-depleted nuclei (○).

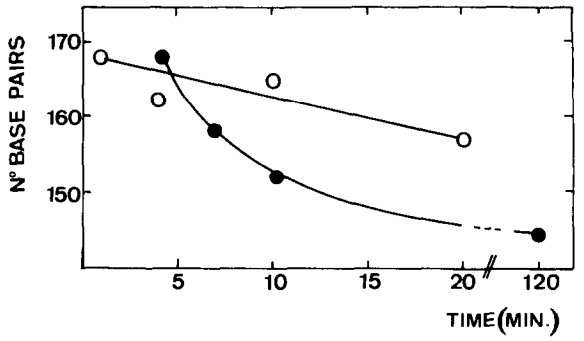

Fig.4. Size of the monomer as a function of digestion time. The values plotted correspond to both whole nuclei (•) and H1-depleted nuclei (○).

(fig.3). In the latter type of nuclei a much higher percentage of monomer DNA is present, but its size diminishes at a lower rate. A slower rate of degradation is also apparent in the dimer and trimer bands, the size of which is given in table 1.

3. Discussion

Histone H1 from calf thymus nuclei is selectively and completely extracted at pH 2.2, in agreement with [14].

The depletion method we have used may appear likely to destroy the nucleosomal organization of chromatin. In fact at acidic pH, DNA in chromatin is denatured. However, when the pH is adjusted back to neutral values, both DNA and chromatin recover their native organization. This pH-dependent structural transition must be different from that found in [15], since we are not able to detect any diffraction at ~2.7 nm. So, the structure of chromatin we have detected at acidic pH is very unlikely to correspond to an unfolded state. Our results are consistent with the persistance in chromatin, at low pH, of a nucleosomal-like structure constituted by core histones and denatured DNA. This observation indicates that DNA in chromatin is able to separate its strands without imposing an irreversible damage on the nucleosomal organization.

Our data on nuclease digestion of H1-depleted chromatin point out the influence of the secondary structure of DNA in determining the nucleolytic cleavage of DNA in chromatin. In fact, assuming that histone H1 protects 20 basepairs of spacer DNA giving rise to a 160 basepair nucleosomal particle [16–20], we would not expect to observe these particles in the absence of histone H1. In spite of this, during the digestion process, we found particles containing ~160 basepairs of DNA, being more resistant to nuclease trimming than those coming from native chromatin. As proposed in [4], nucleases could be a specific probe for the B conformation of DNA, its activity being only secondarily influenced by the degree of coverage of the DNA by histones [21]. Upon acidic treatment the secondary structure of the DNA fragment associated to histone H1 has been modified. This is most probably due to its interaction with the highly charged tails of core histones, since they seem to be uninvolved in the formation of the protein core [22,23]. Through this interaction, DNA could adopt a distorted conformation, being more resistant to nuclease.

However, we have found that when H1 histone has been removed, endonucleolytic cleavage of spacer DNA is faster, as a consequence of the unravelling of the higher order structure of chromatin.

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