THE ISOLATION OF NUCLEOSOMES FROM SALINE-WASHED CHROMATIN

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1. Introduction

Recently a model has been proposed for the structure of chromatin [1], based on a repeating unit of about 200 base pairs of DNA around a core of 8 histone molecules; 2 each of H2A, H2B, H3 and H4. Much of the evidence for this model has come from experiments on digestion of chromatin with micrococcal nuclease [2–4] which produces monomer subunits or nucleosomes [5]. The nuclease digestion is usually carried out on intact nuclei which are subsequently lysed with EDTA [2–4]. Nucleosomes have also been obtained by digesting chromatin [6], but again care has been taken to prevent shearing. Indeed, Noll, Thomas and Kornberg [6] have stated that the chromatin prepared by conventional methods, or methods involving shear, is not native as judged by their criterion. We report in this paper, that as judged by the same criterion, i.e., the size of the DNA produced by micrococcal nuclease digestion, nucleosomes can be made from chromatin which has been prepared by a conventional method which does involve shearing.

2. Materials and methods

All operations, unless otherwise stated, were carried out at 4°C. The tissue used was thymus, removed from rabbits immediately before death and just prior to the experiment. In order to reduce proteolysis, phenylmethylsulphonylfluoride (PMSF) [7] was included in all solutions used for the preparation of the nuclei and chromatin and in the buffer used for the column chromatography.

For comparative purposes, 2 preparations of nucleosomes were carried out simultaneously, one on nuclei and the other on sheared chromatin.

Chromatin was prepared by modifying slightly the method of Butler et al. [8]. 10 g of thymus was homogenised in 100 ml 0.14 M NaCl, pH 7.0, containing 0.2 mM PMSF and 1% isopropanol, using an MSE overhead blender at full speed for 1 min and centrifuging at 1300 *g* for 15 min. This was repeated 4 times, at a reduced blending time of 30 s, to obtain a chromatin pellet, which contained no intact nuclei when it was viewed under the light microscope.

Nuclei were prepared according to Shaw et al. [3]. Using the overhead blender at full speed, thymus was blended for 1 min in the buffer (0.3 M sucrose, 3 mM $CaCl_2$, 5 mM Tris, adjusted to pH 7.3 with cacodylic acid and containing 0.2 mM PMSF, 1% isopropanol) and centrifuged at 250 *g* for 10 min. The pellet was washed once with the same buffer and once with digestion buffer, which differs from the former by having a $CaCl_2$ content of 1 mM and not containing PMSF. Using this procedure thymus nuclei are isolated intact.

The chromatin and the nuclei were suspended in digestion buffer, at a DNA concentration of approx. 3 mg/ml.

Digestion was carried out at 37°C for 10 min, using 200 units of micrococcal nuclease (Worthington Enzymes) per ml of suspension. The reaction was terminated by the addition of 0.1 M EDTA to give a final concentration of 1.7 mM. The EDTA also serves to lyse the intact nuclei. The two suspensions, one from the nuclei and the other from the chromatin, were centrifuged at 1800 *g* for 10 min and the supernatants were retained.

DNA was extracted from part of each supernatant

and the remainders were applied separately to a Bio-Rad A-5M column (previously equilibrated with 0.7 mM EDTA, 10 mM Tris-cacodylate, pH 8, 0.2 mM PMSF, 1% isopropanol) in order to separate monomer nucleosomes from oligomers according to Shaw et al. [3]. DNA was then extracted from the monomer peaks. The method used for the isolation of the DNA was a modified procedure of Mamur [9] employing chloroform and phenol [10].

The DNA samples were subjected to polyacrylamide gel electrophoresis, using 5% gels pH 7.8, according to Loening [11].

3. Results

Before the digestion, the DNA concentration in the suspension of chromatin and in the suspension of nuclei was 3 mg/ml. After digestion and centrifugation, the DNA remaining in the supernatant was approximately 2.5 mg/ml in both cases.

Fig.1 shows the results obtained when the DNA samples from the nuclease digestion of the sheared chromatin and the nuclei are electrophoresed in 5% polyacrylamide gels, and stained with ethidium bromide. It can be seen that these two samples are very similar, both giving discrete bands of DNA. Similarly, fig.2 shows the patterns obtained from the DNA of the monomer peak isolated from the digested chromatin and nuclei, both give a single band of DNA of identical mobility.

No differences were observed between the elution profiles from the Bio-Rad A-5M column of the nuclei and chromatin digests.

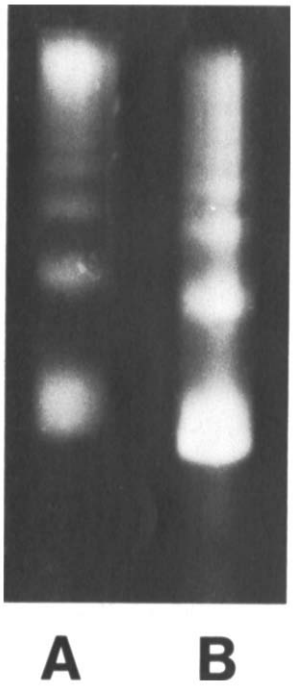

Fig.1. DNA (100 μg) extracted from (A) nuclei, (B) sheared chromatin, after micrococcal nuclease digestion, run in 5% polyacrylamide gels, pH 7.8 for 3.5 h at 5mA/gel and stained with ethidium bromide.

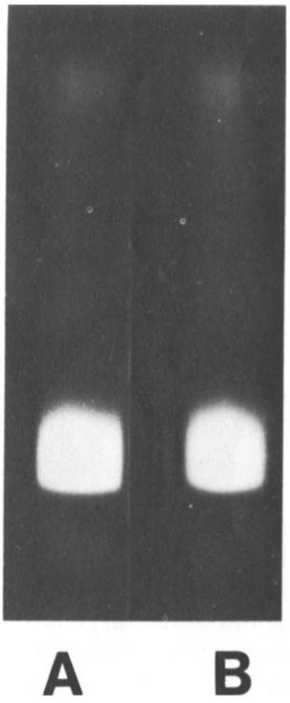

Fig.2. DNA (50 μg) from (A) nuclei and (B) sheared chromatin isolated from the monomer peak, obtained by gel filtration after nuclease digestion. Electrophoresis was in 5% polyacrylamide gels, as in fig.1.

4. Discussion

These results show that the structure of thymus chromatin as judged by the isolation of nucleosomes is not destroyed during its preparation by homogenising with 0.14 M NaCl pH 7.0, and that this chromatin can be digested with micrococcal nuclease, as can intact nuclei, to yield nucleosomes.

It has been reported [6] that the 'native' structure of chromatin is destroyed by shearing forces. However, from the results reported here it can be seen that this is too great a generalisation. It is likely that chromatin in the form of a gel, or at low ionic strength, is more easily sheared than chromatin in 0.14 M NaCl and this may account for the different results. Nevertheless, it should be stressed that chromatin prepared by some 'conventional' methods is adequate for the preparation of nucleosomes.

It is proposed to determine whether the nucleosome structure is still present when 0.14 M NaCl washed chromatin is dialysed to form a gel, and if so, if it is lost when the gel is sheared.

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