PREPARATION AND PURIFICATION OF MONONUCLEOSOME PARTICLES CONTAINING HISTONE H5

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1. Introduction

The nucleosome core particle structure containing 146 basepairs (bp) of DNA and two copies of each histone H2A, H2B, H3 and H4 [1] has been extensively characterized [2–5]. However this structure cannot be considered as the smallest representative subunit of chromatin because it lacks the linker DNA (DNA between two adjacent core particles) and the lysine-rich histone, H1. The isolation and subsequent analysis of a homogeneous nucleosome particle consisting of the core particle, the linker DNA and histone H1 bound to this linker DNA [6] can give further valuable information about the chromatin structure. Such mononucleosome particles obtained to now are very heterogeneous because of the heterogeneity of histone H1 [7] and contamination of the particles with non-histone proteins.

Due to its analogous primary structure, H5 can be considered as a true H1 class variant [8]. During erythropoiesis in birds H5 accumulates up to a H5/H1 ratio of ~3 [9,10]. This means that H5 comprises ~28% of the total histone content of mature erythrocytes [9,10]. Thus relatively large amounts of pure H5-chromatosomes (nucleosome particles containing H5, the octamer histones and ~185 bp DNA) can theoretically be obtained.

These particles are also very interesting because knowledge of their structure can perhaps lead to an explanation of the chromatin condensation and inactivation seen in erythropoietic cells during H5 accumulation [11,12].

We describe here a simple procedure for isolating from chicken erythrocytes pure nucleosome particles containing the octamer histones, lysine-rich histone H5 and ~185 bp DNA referred to as H5-chromatosomes [13]. Using a different approach J. Bode and K. Henco have prepared particles containing a similar length of DNA from erythrocyte chromatin (personal communication). We believe that this H5-containing material will prove to be a valuable tool for the future study of chromatin.

2. Experimental

Fragmented chromatin preparations as well as H1 and non-histone depletions were performed on chicken erythrocytes as in [14]. This depleted chromatin was dialysed overnight against 10 mM Tris–HCl, 0.2 mM EDTA (pH 7.4). A first digestion with 30 units/ml of micrococcal nuclease (Worthington) was performed at 37°C after the addition of 0.6 mM Ca^{2+}. The chromatin had an A_{260} of 20. The digestion was stopped after 7.5 min by the addition of 5 mM EDTA and chilling on ice. The digested chromatin was centrifuged through a linear 10–30% sucrose gradient in 80 mM NaCl, 1 mM sodium phosphate, 0.2 mM EDTA (pH 6.8) for 14 h at 25 000 rev./min in an SW27 Beckman rotor at 5°C. The polynucleosomes were separated from the mononucleosomes and core particles, and dialysed against 10 mM Tris–HCl, 0.2 mM EDTA (pH 7.4).

The second digestion was carried out as above. The time required to obtain a maximum of mononucleosomes and a minimum of core particles was deduced from a pilot digestion and a quick DNA gel electrophoresis as in [15]. In a final step, pure H5-chromatosomes were isolated from a linear 10–30% sucrose gradient in 20 mM NaCl, 1 mM sodium phosphate, 0.2 mM EDTA (pH 6.8) after 16 h centrifugation at 34 000 rev./min at 5°C in an SW41 rotor.

Protein and DNA gel electrophoresis were done as in [14,16].

3. Results and discussion

To characterize by physical methods such as diffraction, NMR, electric dichroism and birefringence, nucleosome particles containing the octamer histones, a lysine-rich histone and a well-defined DNA length resulting from the protection (against nucleases) by these histones, it is necessary to isolate a homogeneous population. With H1 containing nucleosomes this is very difficult to achieve because of the heterogeneity in histone H1 itself [7] and also because of the contamination of the preparations with mononucleosomes carrying non-histone proteins instead of H1, as seen in nucleoprotein-gels [17].

Our H5-chromatosome preparation is based on the possibility to obtain a chicken erythrocyte chromatin selectively depleted of H1 and non-histone proteins and involves two subsequent digestion and purification steps. We began with long fragmented chromatin of chicken erythrocytes solubilised in 80 mM NaCl, 0.2 mM EDTA, 1 mM sodium phosphate (pH 6.8). This chromatin was depleted of H1 and non-histone proteins by the resin AG50W-X2 as in [14]. At this stage we have a mixture of nucleosomes containing H5 and those which have lost their linker DNA-bound proteins. One can enrich the H5 containing nucleosomes by brief nuclease digestion, because the protein-depleted linker DNA is more susceptible to nuclease attack.

For the nuclease digestion the depleted chromatin is first dialysed against a low ionic strength buffer (10 mM Tris–HCl, 0.2 mM EDTA (pH 7.4) to be certain that histone H5 binds tightly to the linker DNA. The core particles (largely originating from digestion of protein-depleted nucleosomes) are separated from the polynucleosomes by sucrose gradient centrifugation (fig.1). As nucleosomes are better separated at high ionic strength [18], 80 mM NaCl sucrose gradients were used. The sucrose gradient was fractionated from the bottom (fig.1) and each fraction was again dialysed against the low ionic strength buffer. A second micrococcal nuclease digestion was done at 37°C to enhance the exo-nucleolytic versus the endonucleolytic action [6]. To find the best digestion time, a pilot digestion on a small volume was performed. At several time intervals a sample was taken and the digestion was

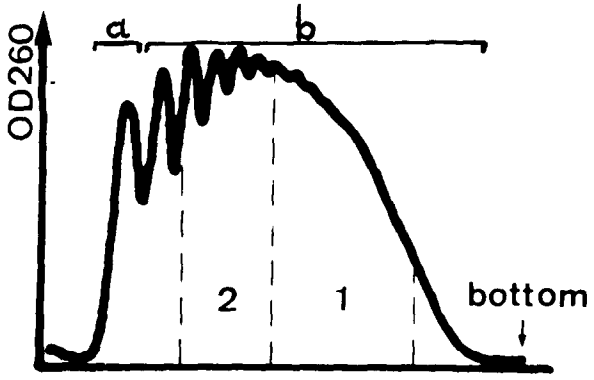


Fig.1. Sucrose profile of H1-depleted chicken erythrocyte chromatin digested with micrococcal nuclease as described in the text. Fractions 1 and 2 were further used in the H5-chromatosome preparation. Letters a and b refer to core particles and polynucleosomes, respectively.

terminated with 5 mM EDTA and chilled on ice. After a quick DNA gel electrophoresis [15] one can determine the optimal time for obtaining a maximum of mononucleosomes and a minimum of core particles. This time was not identical for different preparations and fractions due to the different nucleosome lengths initially present. The H5-chromatosomes were then prepared by digestion of each fraction for the experimentally determined time. In a final step these H5-chromatosomes were isolated from the remaining polynucleosomes by sucrose gradient centrifugation (fig.2). This final sucrose gradient was made in 20 mM NaCl so as to inhibit a possible rearrangement of H5 from mononucleosomes to oligonucleosomes.

The protein gel electrophoresis (fig.3) showed

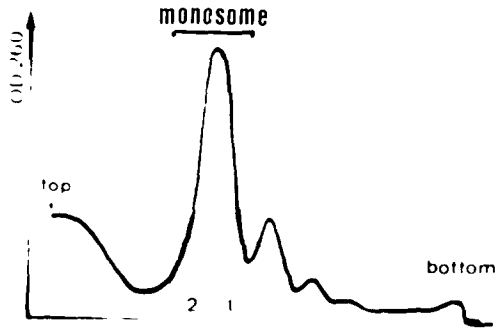


Fig.2. Sucrose profile of fractionated chromatin of fig.1 after the second micrococcal nuclease digestion. Fractions 1 and 2, containing the monosome region were further analysed on protein and DNA gels.

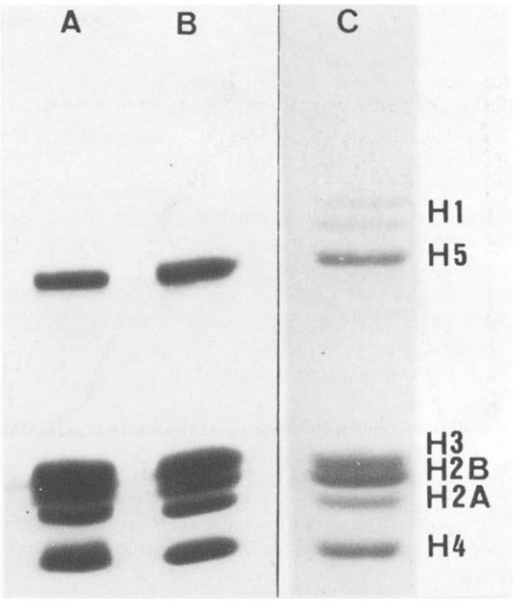
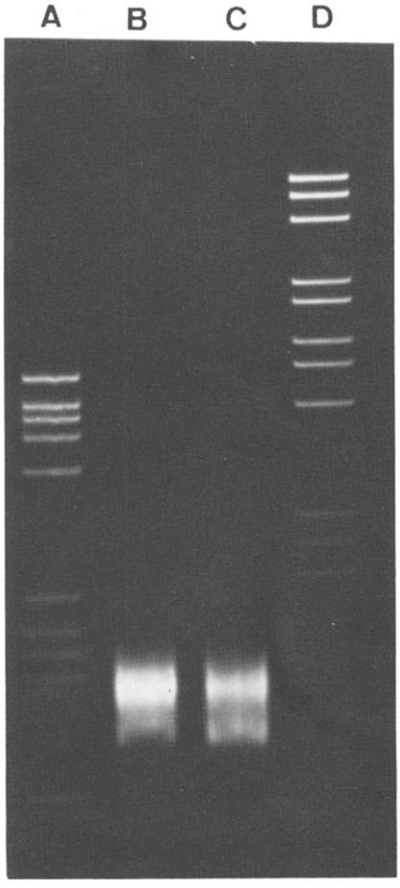


Fig.3. Protein gel electrophoresis of fractions 1 and 2 of fig.2, slots A and B, respectively. Slot C: control run of total chicken erythrocyte chromatin histones.



that each fraction contains only histone H5 plus the core histones. The DNA gel revealed that the isolated H5-chromatosome is composed of a sharp DNA band (fig.4). For the exact length determination we took fraction 2 (slot C in fig.4), consisting of a mixture of core particles and the H5-chromatosome. DNA extracted from this mixture with chloroform/isoamyl alcohol was run on a gel calibrated with *Hae*III restriction fragments of the plasmids pBR322 [19] and PM-2 [20] (fig.5). The negative of fig.5 was scanned.

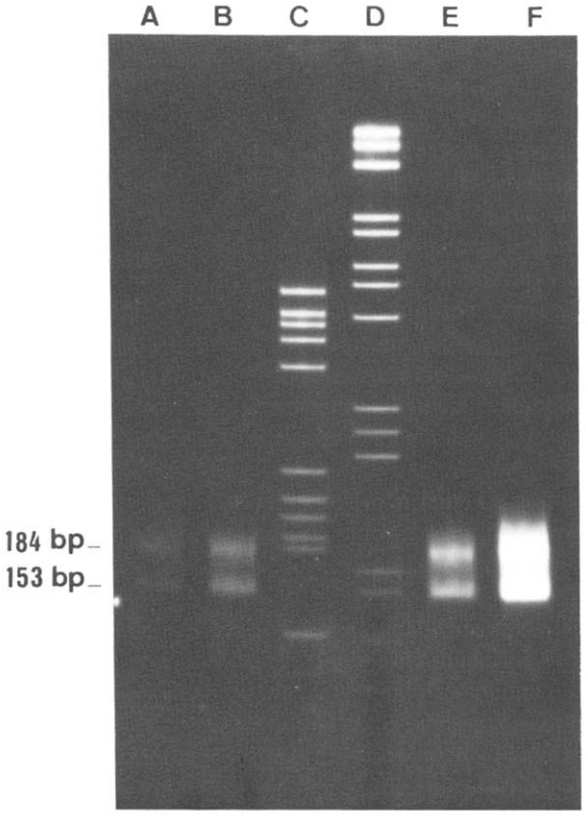


Fig.5. (A) 0.25 μg, (B) 0.5 μg, (E) 1 μg, (F) 2 μg DNA of the monosome fraction as in fig.4 slot C was layered on top of a DNA gel, calibrated with *Hae*III restriction fragments of pBR 322 (C) and PM 2 (D). For the size of the restriction fragments see legend to fig.4.

Fig.4. Fractions 1 and 2 of fig.2 were applied to a DNA gel (slots B and C) with *Hae*III restriction fragments of pBR 322 (slot A) and PM 2 (slot D). Lengths are from top to bottom in basepairs: pBR 322, 587, 540, 504, 457, 434, 267, 234, 213, 192, 184, 124, 123, 104, 89; PM2, 1930, 1820, 1485, 915, 860, 695, 630, 540, 340, 300,277, 167, 152, 120, 95.

The peak maximum of the core particle DNA co-migrated with 153 bp while the peak maximum of the H5 protected DNA fragment co-migrated with 184 bp. The latter finding is quite different from the 166 bp pause found for H1 containing nucleosomes in rat liver [6]. This longer H5-protected DNA length possibly explains the repeat increase from 195 bp for H1-containing chicken liver to 212 bp in the case of mature chicken erythrocytes [21,22].

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