NUCLEODISOME – A NEW REPEAT UNIT OF CHROMATIN REVEALED IN NUCLEI OF PIGEON ERYTHROCYTES BY DNase I DIGESTION

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1. Introduction

It is well known that eucaryotic chromatin is arranged as linear repeat of structural units (nucleosomes) [1–3]. DNA, linking nucleosomes (linker DNA) shows an increased sensitivity to the action of different nucleases. Electrophoresis of DNA fragments isolated from nuclease-digested chromatin reveals a 'ladder' of fragments which are multiple in size to DNA length of an elementary subunit (a nucleosome).

As shown here, the treatment of pigeon erythrocyte nuclei with pancreatic DNase I enabled us to detect a series of DNA fragments the length of which is multiple to the duplicate length of the nucleosome DNA repeat. This implies the presence in avian erythrocytes of a particular DNase I-resistant structure consisting of two nucleosomes (nucleodisomes).

2. Materials and methods

Nuclei were isolated from rat thymus and pigeon erythrocytes as in [4,5]. The hydrolysis of the nuclei with micrococcal nuclease (Worthington) was performed as in [4], and digestion with DNase I (Worthington, free of RNase) was done in a solution comprising 0.3 M sucrose, 10 mM Tris–HCl (pH 7.5), 3 mM $MgCl_2$, 0.1 mM PMSF. After definite time of digestion 1/5 vol. solution containing 0.3 M Tris–HCl (pH 7.4), 10% SDS, 50% glycerol was added and lysate was analyzed electrophoretically.

The DNA electrophoresis was conducted in 2% agarose gel (Sigma) using buffer solution containing 50 mM Tris–acetate, 20 mM Na-acetate, 1 mM EDTA, 0.1% SDS (pH 7.9). Fragments of λ-DNA produced with *Pst*I restriction nuclease were used as molecular mass standards [6].

To obtain chromatin fragments the nuclei after nuclease hydrolysis were lyzed in 1 mM EDTA (pH 7.5) [7,8]. Electrophoresis of chromatin particles was performed in a composite gel containing 2.5% acrylamide and 0.5% agarose [9]. Tris-borate (10 mM) buffer (pH 8.3), 1 mM EDTA was used as an electrophoretic buffer.

3. Results and discussion

The results of electrophoresis in native conditions of DNA fragments isolated from pigeon erythrocyte and rat thymus nuclei treated with micrococcal nuclease or DNase I are presented in fig.1. The digestion of erythrocyte nuclei with micrococcal nuclease yields a usual set of DNA fragments (fig.1a,f). The treatment of erythrocyte nuclei with DNase I, however, results in formation of a ladder of DNA fragments the length of which is multiple to the doubled length of a usual DNA repeat, i.e., of ~430 basepairs (fig.1b–e). The hydrolysis of nuclei from rat thymus with DNase I gives a familiar series of fragments which are multiple, as in the case of micrococcal nuclease action, to the DNA repeat size (fig.1h–j). It means that such extraordinary repeat lengths detected in erythrocyte nuclei after hydrolysis with DNase I is not the consequence of certain experimental conditions but is due to some pecularities of the chromatin structure of pigeon erythrocytes. The data presented in fig.1 also indicate the doubled DNA repeat size revealed after DNase I digestion reflects the structure of the bulk of erythrocyte chromatin since it occurs both at the earliest stages of hydrolysis and in cases when chromatin is heavily fragmented (10% of acid-soluble products of DNA).

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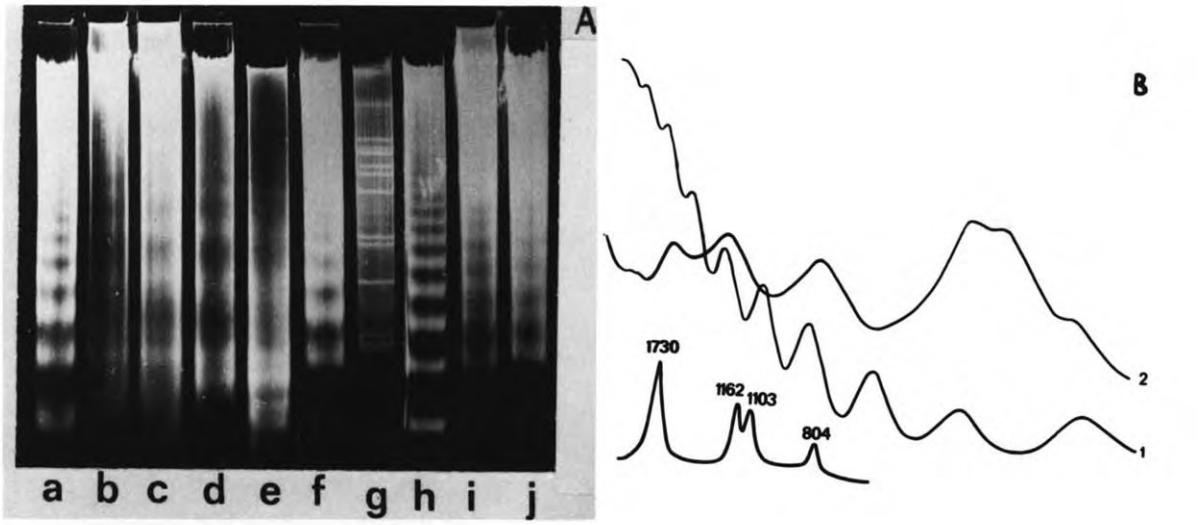

Fig.1. (A) Electrophoresis of DNA from nuclei treated with micrococcal nuclease and DNase I: (a–f) DNA from pigeon erythrocyte nuclei; (h–j) DNA from rat thymus nuclei; (a,f,h) nuclei were digested with micrococcal nuclease, in others nuclei were digested with DNase I. Percentage of acid soluble products of DNA in nuclei digested with DNase I: (b) 0.2%; (c) 0.6%; (d) 3%; (e) 9.4% (g) molecular mass standards. (B) Densitograms of electrophoretic runs from (A). (1) Micrococcal nuclease digest, a; (2) DNase I digest, c; lengths of 4 marker DNA fragments from g.

The fact of an unusual double periodicity of DNA fragmentation is not by itself an evidence of the existence in erythrocyte chromatin of a peculiar structural unit since such a periodicity can appear due to the presence of nuclease-sensitive sites inside the nucleosome core [10]. To prove the existence of the structural units consisting of 2 nucleosomes we performed sucrose density gradient centrifugation and electrophoresis of chromatin particles released into EDTA solution of the DNase I digestion of nuclei.

Fig.2 shows the results of electrophoresis of chromatin particles in a composite gel. As seen from the runs and from the corresponding scans DNase I - solubilized chromatin fragments contain less mono- and trinucleosomes than dinucleosomes and tetranucleosomes (as compared to micrococcal nuclease digest). Taking into consideration the capability of DNase I to generate formation of single-stranded DNA during chromatin digestion [11] the accumulation of ethidium bromide-staining material in the region of trinucleosomes may be accounted for by the irregular destruction of higher oligonucleosomes (fig.2).

Sucrose density gradient centrifugation of chromatin particles enables us to confirm electrophoretic observations that the dinucleosomes are the predominant fraction in DNase I-digested chromatin (fig.3).

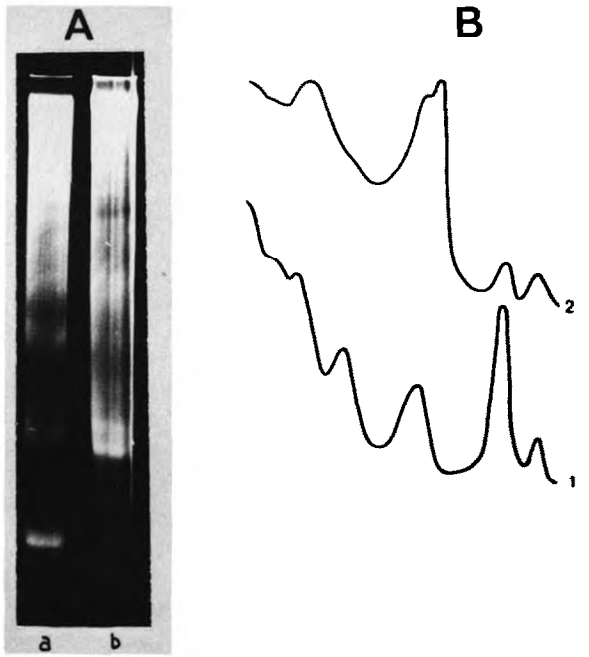

Fig.2. Electrophoresis of chromatin particles from pigeon erythrocyte nuclei digested with micrococcal nuclease and DNase I. (A) Electrophoresis in a composite 0.5% agarose–2.5% polyacrylamide gel: (a) micrococcal nuclease; (b) DNase I (1.1% of DNA acid-soluble products). (B) Scan of (a) and (b) from A.

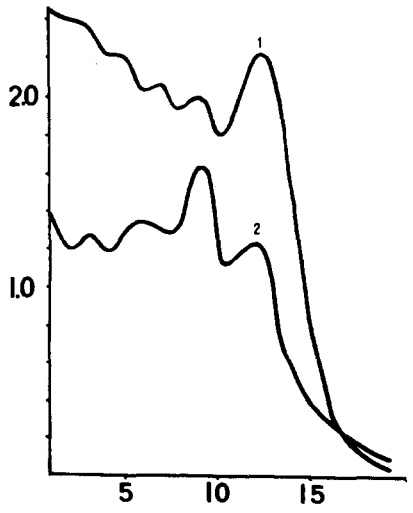

Fig.3. Sucrose density gradient centrifugation of chromatin particles of DNase I-treated erythrocyte nuclei. Chromatin was layered onto a 5–20% linear sucrose gradient and centrifuged for 22 h at 25 000 rev./min: (1) micrococcal nuclease digest; (2) DNase I digest.

Thus it may be concluded that pigeon erythrocyte contains a particular structural unit consisting of two nucleosomes. This unit may be called as nucleodisome. The nucleodisome is characterized by a greater resistance of internal linker DNA as compared to the external ones owing to which DNase I yields very few particles with an odd number of nucleosomes (fig.2, fig.3). As seen from fig.1 the nucleodisome (dimer) population is heterogeneous. The heterogeneity occurs along the length of the DNA fragment, its length being reduced with the increase of the degree of digestion.

It is most likely that the nucleodisome organization is typical of very condensed chromatin which is characteristic for pigeon erythrocytes. Since erythrocyte histones comprise a specific histone fraction H5 it cannot be rule out that it is responsible for the formation of nucleodisomes.

Note added

After this work was finished Arceci and Gross (Dev. Biol. 80, (1980) 210–224) described similar doubled DNA fragmentation with DNase I in sea urchin sperm nuclei.

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