NUCLEASE DIGESTION OF RECONSTITUTED CHROMATIN

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1. Introduction

Nuclease digestion experiments have recently provided evidence for the existence of a periodic structure in chromatin [1–4]. It is not clear, whether and to what extent the periodic structure re-forms when dissociated chromatin is reconstituted.

The procedures for dissociation and reconstitution of chromatin may be divided into two groups according to the presence [5–9] or absence [10–12] of urea. In this work we study the degradation products with micrococcal nuclease (EC 3.1.4.7) and DNAase I (EC 3.1.4.5) of chromatin reconstituted in the presence and in the absence of urea. Chromatin reconstituted by a salt step gradient only give discrete fragmentation patterns, suggesting that these preparations possess a secondary structure similar to that of untreated chromatin. The presence of urea during the reconstitution procedure abolishes the specificity of enzyme fragmentation of chromatin DNA.

2. Materials and methods

Isolation of structured chromatin from Guerin ascites tumour, chemical analyses, digestion with micrococcal nuclease and DNAase I, determination of acid soluble products, isolation of DNA and its electrophoretic fractionation and determination of the template activity of chromatin in vitro with RNA polymerase from *Escherichia coli* (EC 2.7.7.6) have been described in detail elsewhere [13–15]. The dissociation-reconstitution procedures used were the following. Chromatin (8 A_{260}-units/ml in 2 mM Tes buffer, 10 μM EDTA (pH 7.8) containing 0.1 mM phenylmethylsulfonyl fluoride) was buffered with Tris–Cl (10 mM final concentration) at pH 7.8 and dissociated by addition of solid NaCl to 2.5 M (salt method) or solid NaCl and solid urea to 2.5 M and 6 M respectively (salt-urea method). The samples with final concentration of 3 A_{260} units/ml were left overnight at 4°C and then dialysed according to the schedule in table 1.

The chemical composition of reconstituted chromatins (total protein/DNA, histone/DNA) did not differ significantly from that of control chromatins.

3. Results

Digestion with micrococcal nuclease to 15–20% acid soluble products converts DNA in the salt-reconstituted chromatin into discrete fragments superimposed on a heterogeneous background (fig.1B).

Table 1

Time (h)	Outer dialysis solution		
	Volume (× the sample volume)	Composition	
		Salt method	Salt-urea method
2	10	Solution A	Solution B
2	10	Solution A	Solution B
2	10	Solution A	Solution B
18	20	Solution A	Solution A
3	20	Solution A	Solution A
3	20	Solution A	Solution A
18	50	Solution C	Solution C

Solution A: 10 mM Tris–Cl, 10 μM EDTA (pH 7.8)
Solution B: 10 mM Tris–Cl, 10 μM EDTA, 6 M urea (pH 7.8)
Solution C: 2 mM Tes, 10 μM EDTA (pH 7.8)

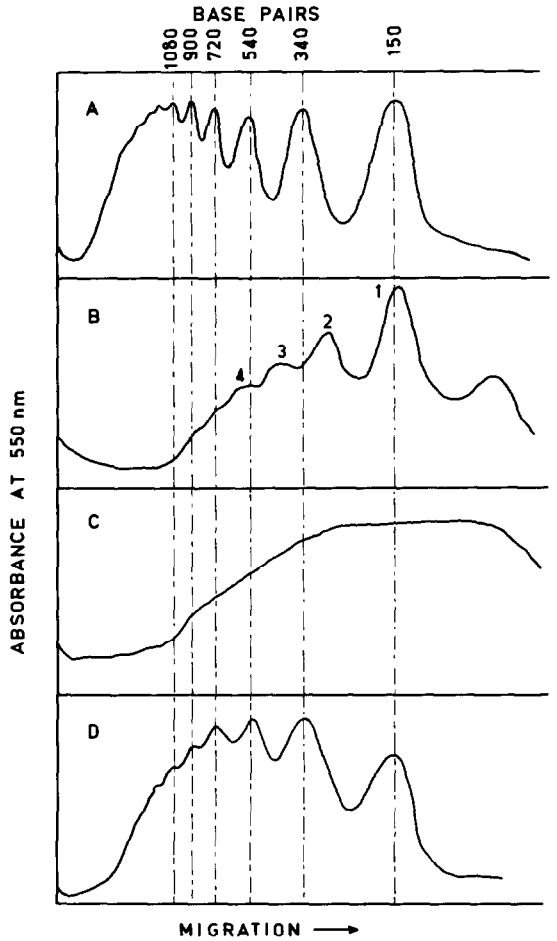

Fig.1. Electrophoretic profiles in 2.5% polyacrylamide gel under non-denaturing conditions [13] of DNA isolated from chromatins digested with micrococcal nuclease. (A) Control chromatin, 10% acid soluble products; (B) Chromatin reconstituted by the salt method, 18.8% acid soluble products; (C) Chromatin reconstituted by the salt-urea method, 22.6% acid soluble products; (D) Chromatin treated with 6 M urea and dialysed back to buffer, 6.2% acid soluble products.

The mean values of their sizes (table 2) are close to the values of the series mol. wt. = 150 $n - s$, where n is the number of the peak (fig.1B) and s is the length of the 'spaced' DNA (10–40 base pairs). These sizes are smaller than those of DNA fragments isolated from control chromatin digested in the same way [13] (fig.1A), which are multiples of 180 base pairs.

Digestion of salt-reconstituted chromatin by DNAase I yields the well known series of fragments, multiples of 10 nucleotides [3] observed under denaturing conditions (fig.2A), identical to the DNAase I digestion pattern of untreated chromatin [15]

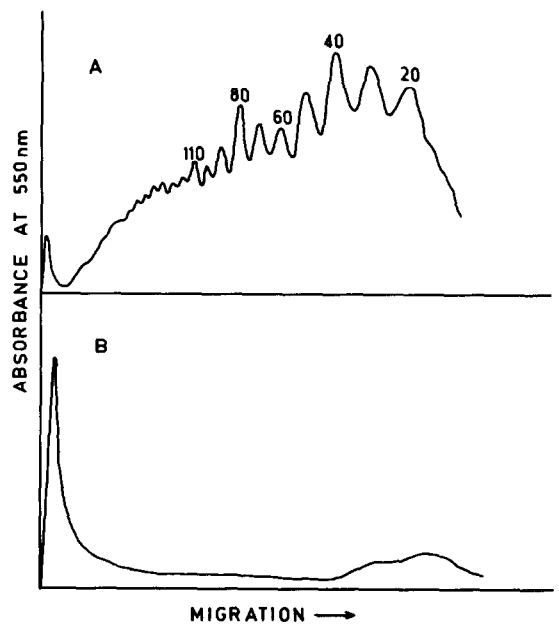

Fig.2. Electrophoretic profiles in 7.5% polyacrylamide gel under denaturing conditions [15] of DNA isolated from chromatin reconstituted by (A) the salt method, and digested with DNAase I to 34.5% acid soluble products; (B) the salt-urea method, and digested with DNAase I to 28.4% acid soluble products. The numbers show the chain length in nucleotides.

Table 2
Molecular weights in base pairs of the DNA fractions isolated from chromatin reconstituted by the salt method and digested with micrococcal nuclease

Fraction No[a]	1	2	3	4
Base pairs ± σ	142 ± 7	260 ± 18	386 ± 35	about 580

[a]Fraction numbers as in fig.1B.
σ is standard deviation of the mean from 4 experiments.

In contrast, the chromatin reconstituted by the salt-urea method yields a random size distribution of DNA when digested with micrococcal nuclease (fig.1C). Treatment of control chromatin with 6 M urea alone prior to digestion and dialysis back to buffer does not alter the spacing of the fractions, although some increase of the heterogeneous background is observed (fig.1D).

DNAase I digestion of chromatin reconstituted by the salt-urea method fails to produce discrete fragments (fig.2B). While part of DNA is hydrolysed to acid soluble products, the remaining DNA is found in a fraction of relatively high molecular weight.

The template activities of all reconstituted chromatins, regardless of the method of reconstitution, are close to that of untreated chromatin [13] i.e. about 5% of the template activity of free DNA.

3. Discussion

We favour the following explanation of the results described above. It is known that in the chromatin the histones (except H1) are maintained by apolar interactions in the form of specific complexes [11]. Each of these complexes interacts with about 150 base pairs of DNA, forming nucleosomes interspersed with bridges of about 30 base pairs [16,17]. In the salt method for reconstitution, these histone complexes dissociate from DNA as a whole, being stable in high salt [11]. On the other hand, it has been suggested that H1 is bound to the 'spacer' DNA [16]. It may be speculated that if H1 determined the regular spacing of the nucleosomes along DNA, then upon lowering the ionic strength the complex of the non-H1 histones would re-bind DNA at salt concentrations that would keep H1 still in solution and would be packed along DNA in a random fashion with variations in the length of the 'spacer' DNA larger that those occuring in the untreated chromatin. Upon digestion with micrococcal nuclease the longer 'spacers' would be affected first, while regions with closely packed nucleosomes would remain intact, yielding multiples of about 150 base pairs. This is to be expected with preparations, which have heterogeneously spaced nucleosomes and could explain the finding [16] that the large DNA fragments decrease in size in the course of digestion with micrococcal nuclease more than expected from digesting away the 'spacer' DNA at the two ends.

When chromatin is dissociated by salt and urea, the specific interactions between the histones are abolished. Lowering the salt concentration in high urea would keep the proteins as single polypeptide chains devoid of much of their secondary structure [18]. It is conceivable that under these conditions the proteins will rebind DNA at random and the information inherent to the specific protein–protein interactions will be lost. The effect of urea observed by us is in agreement with the X-ray data of Boseley et al. [8].

In summary, our experiments show that the salt-urea method of reconstitution gives no structural fidelity, while the products of reconstitution in the absence of urea bears some structural resemblance with the untreated material. It appears also that the organization of chromatin in the form of nucleosomes is not necessary for the suppression of the reaction catalysed by *E. coli* RNA polymerase.

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