Persistence of the ten-nucleotide repeat in chromatin unfolded in urea, as revealed by digestion with deoxyribonuclease I

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ABSTRACT

It is shown by enzymatic digestion of chromatin from rat liver or Guerin ascites tumour (GAT) that treatments, which abolish the 180 base pair repeat, as revealed by digestion with micrococcal nuclease (shearing in salt solutions of medium ionic strength, sonication, fixation with formaldehyde in the presence of 5 M urea), have little effect on the 10 nucleotide repeat, observed in deoxyribonuclease I digests.

INTRODUCTION

Digestion of whole nuclei (1,2) or isolated chromatin (3,4) with micrococcal nuclease (E.C.3.1.4.7) in its initial stage generates a series of particles, containing discrete sizes of DNA, multiples of 180 base pairs. Digestion with DNase I produces single stranded DNA fragments, revealed under denaturing conditions, which are multiples of 10 nucleotides (5). The latter has been assumed to reflect the substructure of nucleosomes (5).

In this communication we present evidence that the 10 nucleotide repeat persists under conditions where the 180 base pair repeat is abolished.

MATERIALS AND METHODS

"Structured" and "salt" chromatins were isolated from rat liver or GAT as described elsewhere (4). DNase I was a product of Worthington, N.J., U.S.A. Micrococcal nuclease was obtained from Sigma. All chemicals were of analytical grade. Urea was deionized by ion exchange.

Chromatin (2 A₂₆₀-units/ml in 2 mM TES buffer (pH 7.8),

10 μ M EDTA, 0.1 mM phenylmethylsulfonyl fluoride was digested with micrococcal nuclease in the presence of 0.3 mM CaCl_2 or with DNase I in the presence of 0.1 mM MgCl_2 . In both cases the incubation was for 60 min at 37°C. The reaction was stopped by addition of EDTA in excess. Determination of acid soluble products and isolation of DNA were performed as described elsewhere (4). DNA was analysed by electrophoresis in 2.5% non-denaturing polyacrylamide gel (4) or in 7.5% gel under denaturing conditions (6).

RESULTS AND DISCUSSION

In a previous work we have shown that no discrete fragmentation with micrococcal nuclease is observed in the initial digestion stage (less than 10% acid soluble products) of chromatin prepared by a procedure which includes homogenization in the presence of 0.15 M and 0.35 M NaCl ("salt" chromatin) (4). These observations, together with the results of other

Fig. 1. Electrophoretic profile in 7.5% denaturing polyacrylamide gel of single stranded DNA fragments isolated from "salt" chromatin, digested with DNase I as described in Methods to 29% acid soluble products.

authors (7,8) suggest that in such chromatins the periodic structure of 180 base pairs and multiples has been damaged.

Fig. 1 shows the electrophoretic pattern of DNA from rat liver "salt" chromatin, which is identical with the pattern obtained with whole nuclei (5) and "structured" chromatin (6). Similar results were obtained with "structured" chromatin that had been sonicated (data not shown). DNA isolated from the sonicated chromatin had an average size of 600 base pairs. Digestion of such chromatins with micrococcal nuclease to acid soluble material between 5 and 10% failed to reveal discrete fragmentation of DNA, i.e. the ultrasound was detrimental to the subunit structure of chromatin. The same chromatin samples, when digested with DNase I to acid soluble products between 15 and 20% yielded the characteristic pattern of single stranded DNA fragments, multiples of 10 nucleotides, identical to that shown in Fig. 1.

These results suggest that the 10 nucleotide repeat may exists in the absence of secondary structure in chromatin.

To substantiate this possibility we carried out digestions with both micrococcal nuclease and DNase I of "structured" chromatin, fixed with formaldehyde in the presence of 5 M urea. The unfolding effect of 5 M urea on chromatin is known from the work of Bartley and Chalkley (9). It has also been shown that formaldehyde fixation in the presence of urea abolishes the periodic structure of chromatin, as judged from x-ray diffraction experiments and electron microscopy (10).

Structured chromatin from Guerin ascites tumour was fixed with formaldehyde in the presence of 5 M urea as described in (10): chromatin in 2 mM Tes, 10 μ M EDTA (pH 7.8) at a concentration of 4 A_{260} units per ml was mixed at 0°C with an equal volume of 10 M urea, previously deionized and buffered with 2 mM Tes at pH 7.8. After 15 min in ice, 30% formaldehyde (pH 7.0 adjusted with NaHCO_3) was added to a final concentration of 1%, the mixture was kept in ice for 30 min and dialysed against 3 changes of 100 vol. each of 2 mM Tes, 10 μ M EDTA (pH 7.8) during at least 48 h at 4°C. After this treatment the histones could be extracted quantitatively with 0.25 N H_2SO_4

for 40 min at 0°C, but they failed to enter 15% urea-acetic acid gel (11). If fixation was carried out with 10% formaldehyde in the presence of 5 M urea, the histones became unextractable under the same conditions, but this did not affect the digestion results.

Fig. 2 A shows the normal micrococcal nuclease digestion pattern of untreated chromatin, consisting of discrete fragments, multiples of 180 base pairs. This pattern is not observed with chromatin fixed in urea (Fig. 2 B) which is consistent with the observations of Carlson et al. (10).

Fig. 2. Electrophoretic profile of DNA fragments isolated from a micrococcal nuclease digests of untreated chromatin (A) and chromatin fixed with formaldehyde in the presence of 5 M urea (B). In both cases the digestions was carried out to 5% acid soluble products. Electrophoresis was in 2.5% polyacrylamide gel under non-denaturing conditions.

Nevertheless, the 10 nucleotide repeat survives, as demonstrated in Fig. 3 A.

Fig. 3. DNA fragments from chromatin fixed with formaldehyde in the presence of 5 M urea and digested with DNase I to 16.2% acid soluble products (A) and from chromatin, digested with DNase I in the presence of 5 M urea to 18.7% acid soluble products. 7.5% polyacrylamide gels, denaturing conditions.

While this study was in progress, Jackson and Chalkley (12) reported that micrococcal nuclease was active in the presence of 5 M urea. This stimulated us to test the activity of DNase I under the same conditions. It was found that the enzyme was

active and exhibited its normal requirement for Mg^{++} , but its specific activity was about 20 times lower.

Incubation of chromatin with DNase I in the presence of 5 M urea resulted in the normal digestion pattern (Fig. 3 B), showing the 10 nucleotide repeat.

The persistence of the 10 nucleotide repeat in chromatin fixed in the presence of urea or digested in urea may mean that either this type of periodicity is not dependent on the secondary folding of the deoxynucleoprotein fiber (if we assume that urea causes a complete unfolding) and in this case it is determined by the direct interactions between histones and DNA, or urea does not extend the chromatin completely and some secondary structural features remain intact to determine the observed specificity of DNA fragmentation with DNase I. The latter possibility is suggested by the finding of Jackson and Chalkley (personal communication), that one can observe a specific cleavage of chromatin DNA with micrococcal nuclease in the presence of 5 M urea into approximately 200 base pairs and multiples. Our results, however, definitely point out that the 10 nucleotide repeat may exist independently of the 180 base pair repeat in the sense that they are determined by different structural parameters of chromatin.

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