# Relaxation of chromatin structure upon removal of histones H2A and H2B

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Modification of chromatin from chicken erythrocytes with dimethylmaleic anhydride is accompanied by its solubilization and the dissociation of histones H1, H5, H2A and H2B. Histone H1 is the first to dissociate and H5 the last. After regeneration of the modified amino groups, residual chromatin preparations with different histone composition were studied by circular dichroism and thermal denaturation. In addition to the effects produced by the lack of histones H1 and H5, both techniques show a substantial relaxation of chromatin structure induced by the loss of histones H2A and H2B, which appear to play an important role in the superhelical folding of DNA.

*Chromatin structure* *Histone H2A* *Histone H2B* *Histone dissociation* *DNA folding* *Dimethylmaleic anhydride*

## 1. INTRODUCTION

Chromatin is basically formed by the interaction of double-stranded DNA with the positively charged histones. Although a large amount of information on the structural unit of chromatin, the nucleosome, is now available [1–3], more remains to be discovered about the contribution of particular histone–histone and histone–DNA interactions to the overall chromatin structure. The study of chromatin preparations devoid of particular histone components might help in understanding of the structural contribution of each component.

Treatment of different nucleoprotein particles with dimethylmaleic anhydride, a reversible reagent for protein amino groups, is frequently accompanied by dissociation of specific components [4–6]. We have shown that modification of isolated nucleosomes with this reagent produces release of histones H1, H5, H2A and H2B, and the formation of residual particles containing H3 and H4 [7]. Structural studies of these residual particles and the corresponding nucleosomes reconstituted in the presence of the released histones are consistent with the participation of histones H2A and H2B in the folding of a substantial fraction of nucleosomal DNA [8].

This paper studies the structural properties of chromatin deficient in histones H1, H5, H2A and H2B, obtained after treatment of native chromatin with dimethylmaleic anhydride. The results show a substantial relaxation of chromatin structure in the absence of histones H2A and H2B.

## 2. MATERIALS AND METHODS

### 2.1. *Preparation of chromatin*

Nuclei were obtained from chicken erythrocytes after lysis of the cells in a buffer solution containing 0.5% of the nonionic detergent Nonidet P40 [9]. The nuclei, suspended in 5 mM Tris–HCl (pH 7.5), 2 mM $MgCl_2$ and 66% (v/v) glycerol (4 ml containing $4 \times 10^9$ nuclei/ml), were stored at −20°C. They were lysed by addition of 0.25 mM EDTA (pH 8.2, 100 ml), and centrifuged for 5 min at 4°C and 12000 rpm in an SS34 rotor. The sediment was suspended in 100 ml EDTA solution and centrifuged under the same conditions. This pro-



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cess of suspension and centrifugation was performed 4 more times. The sediment obtained after the last centrifugation was suspended in 50 ml EDTA solution, and subjected to mild stirring at 2–4°C for 24 h. DNA was extracted from chromatin according to [10].

### 2.2. *Modification of chromatin with dimethylmaleic anhydride*

Chromatin (0.25 mg DNA/ml), in 10 mM Hepes ($K^+$) (pH 8.2), 5 mM EDTA and 0.1 mM phenylmethylsulfonyl fluoride (PMSF), was treated at room temperature with the desired amount of dimethylmaleic anhydride in dioxane. During reaction, the chromatin suspension was stirred and the pH maintained at 8.2 by addition of 0.5 mM KOH with a pH-stat assembly (Radiometer, Copenhagen). The hydrolyzed reagent was eliminated by overnight dialysis at 2–4°C against 10 mM Tris–HCl (pH 8.2), 5 mM EDTA and 0.1 mM PMSF. After dialysis, the preparation was centrifuged for 30 min at 2°C and 40000 rpm in an SW50 rotor to separate the insoluble chromatin fraction. The supernatant was centrifuged under the same conditions for another 17 h to separate the released proteins from the sediment containing the residual chromatin, which was suspended in 0.25 mM EDTA (pH 8.2). When required, the modified amino groups were regenerated by dialysis of the reagent-treated preparations at 2–4°C for 6 h against 10 mM maleate (pH 6.0), 5 mM EDTA and 0.1 mM PMSF.

### 2.3. *Electrophoretic analysis of histones*

Samples were dialyzed against 0.5% acetic acid and lyophilized prior to electrophoresis, which was conducted on polyacrylamide gels prepared from 15% acrylamide and containing 0.1% SDS [11]. Histones in the gels were stained with a solution containing 0.05% Coomassie blue, 0.5% (v/v) acetic acid and 10% (v/v) ethanol.

### 2.4. *Circular dichroism*

Spectra were obtained with a Mark III dicrograph (Jobin-Yvon) using quartz cells with 1.0 cm optical path. Ellipticity is expressed on the basis of DNA nucleotide residue concentration. Ellipticity was calculated and the spectra were plotted with a minicomputer HP9815A connected to an HP7225A plotter through a HP-IB interphase. The chromatin preparation (50 $\mu$g DNA/ml) was studied in 0.25 mM EDTA (pH 8.2) at room temperature, and a sensitivity of 2–5 $\times$ $10^{-6}$ $\Delta A$/mm was used.

### 2.5. *Thermal denaturation*

After dialysis of the samples against 0.25 mM EDTA (pH 8.2), denaturation profiles were obtained with a Beckman DU-8 spectrophotometer equipped with a TM.S module, using a heating rate of 0.5°C/min. Absorbance at 260 nm was registered at 1°C intervals between 30 and 102°C using a slit of 0.5 nm. Hyperchromicity values and the derivative, $dH/dt$, were obtained from the registered data by using an HP-85 minicomputer as in [12]. The derivative denaturation curves were resolved into component thermal transitions by Gaussian curve fitting with a BMDP-3R nonlinear regression program run on an IBM 360-65I computer provided with an operating system OS/360. $T_m$, the transition midpoint, is the temperature of maximum $dH/dt$ for each transition.

## 3. RESULTS AND DISCUSSION

Treatment of chromatin from chicken erythrocytes with the reagent for protein amino groups dimethylmaleic anhydride is accompanied by its progressive solubilization. Untreated chromatin when centrifuged for 30 min at 40000 rpm in an SW50 rotor is quantitatively pelleted. After treatment with the reagent, substantial amounts of chromatin which increase with the extent of treatment remain in the supernatant. The proportion of chromatin solubilized ranges from 50% (0.75 mg dimethylmaleic anhydride/mg DNA) to 90% (2.0 mg dimethylmaleic anhydride/mg DNA). The chromatin not solubilized by the treatment maintains the protein and DNA ratio and the histone composition of the untreated preparation. We have directed our attention to studying the fraction of solubilized chromatin.

Centrifugation of the chromatin fraction solubilized by treatment with dimethylmaleic anhydride produces a supernatant containing the released histones and a sediment, residual chromatin, which includes all the DNA and part of the histones. Fig.1 shows the electrophoretic pat-

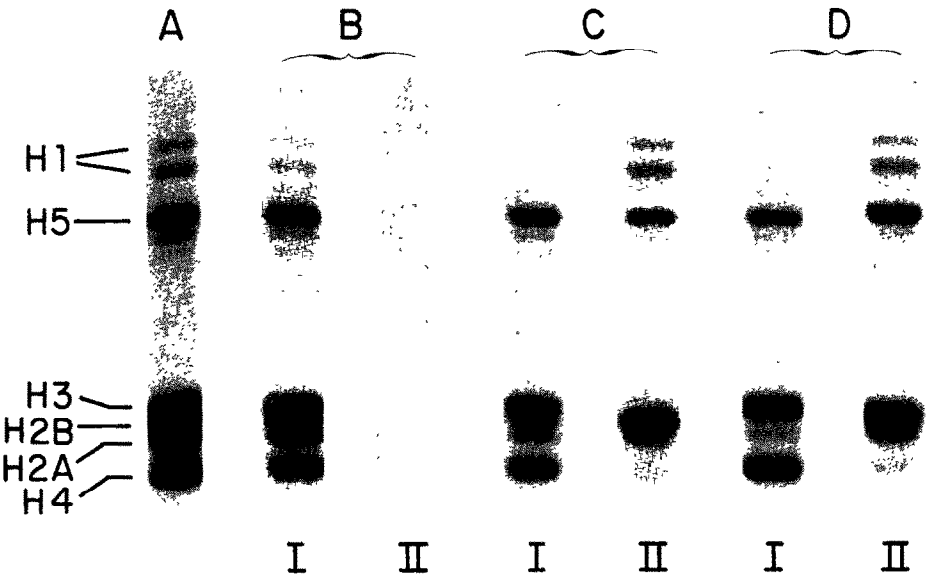


Fig.1. Electrophoresis of the histones released and of those present in the residual chromatin for different treatments. Chromatin was treated with 0.75 (B), 1.5 (C) and 2.0 (D) mg dimethylmaleic anhydride/mg DNA. Samples I correspond to residual chromatin, and samples II to the released histones. A is a control of untreated chromatin. Fractions I and II for each treatment correspond to the same amount of untreated chromatin.

terns of the histones released and of those present in the residual chromatin, for preparations treated with different amounts of reagent. Histones H3 and H4 remain bound to DNA even at the highest treatment used. Of the released histones, H1 is the first to dissociate, followed by H2B and H2A, and lastly by H5.

The structure of the residual chromatin, after regeneration of the modified amino groups, was studied by circular dichroism and thermal denaturation. Fig.2 shows the circular dichroism spectra of residual chromatin preparations obtained by treatment with different amounts of dimethylmaleic anhydride. The ellipticity values between 260 and 300 nm increase with the extent of treatment, while the wavelength at the crossover point remains practically unchanged. The circular dichroism spectrum of chromatin or isolated nucleosomes between 250 and 320 nm can be resolved into two components, according to [13,14]: one corresponds to the secondary structure of free DNA (B-DNA), the other is a negative band attributed to the condensation of B-DNA into an asymmetric tertiary structure. If the considerations in [13,14] are used to calculate the number of nucleotide base pairs that maintain a DNA tertiary arrangement in the different preparations of residual chromatin, the values shown in table 1 are obtained. The residual chromatin with the lowest content of histones

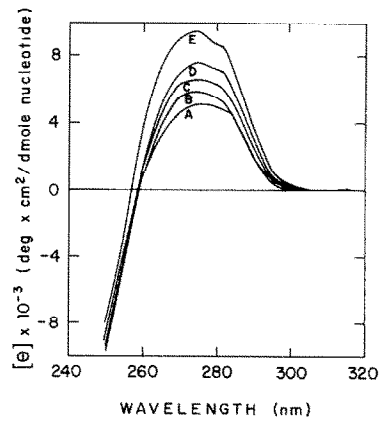


Fig.2. Circular dichroism spectra of residual chromatin obtained with different treatments. Residual chromatin was obtained by treatment with 0.75 (B), 1.5 (C) and 2.0 (D) mg dimethylmaleic anhydride/mg DNA. These preparations are identical to samples B-I, C-I and D-I used for electrophoresis (fig.1). The spectra of untreated chromatin (A) and the corresponding free DNA (E) are also included.

Table 1

Circular dichroism parameters for different residual chromatin preparations

| | DNA | Native chromatin | Residual chromatin | | |
|---|---|---|---|---|---|
| | | | B | C | D |
| $[\theta]_{275}$ | 9300 | 5100 | 5800 | 6600 | 7700 |
| $[\theta]_{275} - [\theta]_{275}$ DNA | | −4200 | −3500 | −2700 | −1600 |
| Condensed DNA (bp)/210 bp DNA | | 140 | 117 | 90 | 53 |

Table 2

Transition temperatures and relative areas of thermal transitions in native and residual chromatin

| | Native chromatin | | | Residual chromatin | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | B | | | C | | | D | | | |
| Thermal transitions | b | c | d | b | c | d | b | c | d | a | b | c | d |
| $T_m$ (°C) | 71.7 | 75.7 | 87.3 | 67.6 | 73.3 | 84.8 | 61.0 | 72.8 | 84.4 | 54.8 | 60.5 | 67.9 | 83.7 |
| Hyperchromicity (%) | 29.8 | 4.9 | 65.3 | 7.8 | 42.6 | 49.6 | 36.5 | 21.0 | 42.5 | 29.8 | 13.7 | 21.5 | 35.0 |

H2A.H2B has an estimate of only 53 nucleotide base pairs in the condensed structure per nucleosomal repeat, as compared to 140 for untreated chromatin, and to 113 for chromatin devoid of histones H1 and H5 [14]. These calculations seem to indicate that the loss of histones H2A.H2B causes a large unfolding of DNA, in agreement with the results obtained with isolated nucleosomes [8].

The thermal denaturation profiles of different preparations of residual chromatin are shown in fig.3. The number of nucleotide base pairs melting at the highest temperature transition, as well as the midpoint temperature of the different transitions, decreases with increasing modification (table 2). The DNA of native chromatin melting in transition b seems to correspond to linker DNA, while that in transition d would correspond to nucleosomal core DNA [15]. The destabilization of DNA in the residual chromatin, as compared to native, is much larger than would be expected from the lack of histones H1 and H5 [15]. In the residual chromatin with the lowest H2A.H2B content, the proportion of DNA highly stabilized against thermal denaturation (74 bp per 210 bp DNA) is larger than that of DNA in a condensed tertiary structure as estimated by circular dichroism (53 bp per 210 bp DNA). This result is not surprising in view of the different structural properties detected by the two methods, and the uncertainty implicit in the evaluation of the circular dichroism data.

In conclusion, both circular dichroism and thermal denaturation show a substantial relaxation of

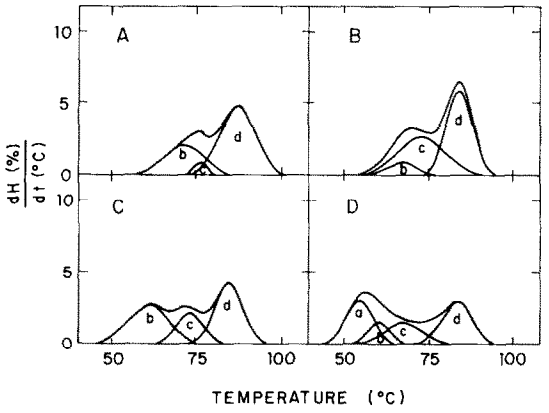


Fig.3. Thermal denaturation profiles of residual chromatin obtained with different treatments. The preparations are identical to those identified by the same letters in fig.2. In addition to the experimental curves, the resolved Gaussian transitions are included. In profile D, an additional Gaussian transition was introduced to obtain a better fit.

chromatin structure induced by the loss of histones H2A and H2B, which stresses the important structural contribution of these histones.

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