CHROMATIN CONFORMATION AS AFFECTED BY SHEARING AND BY THE REMOVAL OF A PORTION OF NON-HISTONE PROTEINS

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1. Introduction

Circular dichroism (CD) spectrum of unsheared cerebral neurone-rich chromatin, as compared with that of liver chromatin, is characterised by its higher mean residue ellipticity at 260–300 nm, particularly at the 270 nm region [1,2]. It has also been shown that neurone-rich chromatin is provided with an unusual wealth of high mol. wt non-histone proteins [3]. In an attempt to analyse a possible correlation between these two findings, we have noticed that the spectrum of chromatin is profoundly affected by the CD spectrum of chromatin is profoundly affected by the removal of a portion of non-histone proteins and also by shearing.

2. Materials and methods

2.1. *Preparation of chromatin*

Cerebral and liver nuclei from the adult guinea pig were obtained as the pellet at the bottom of 2.0 M sucrose–1.5 mM $CaCl_2$ [1,2], and stored frozen at –80°C in 0.32 M sucrose–1.5 mM $CaCl_2$. Further fractionation of cerebral nuclei into neurone-rich and oligodendroglial populations was not carried out. They were washed with 0.5% Triton X-100 in 0.32 M sucrose–1.5 mM $CaCl_2$ immediately before preparing chromatin. Chromatin was prepared essentially as described by Shaw and Huang [4].

2.2. *Dissociation of chromatin with 0.3 M NaCl*

Freshly prepared chromatin was dialysed overnight against 0.3 M NaCl–0.01 Tris·HCl buffer, pH 8, containing 0.1 mM phenylmethylsulphonyl fluoride (PMSF) to minimise the proteolytic degradation [5]. The salt-treated chromatin was then centrifuged at 100 000 *g* for 2 h, and the pellet was suspended by homogenisation in 0.01 M Tris·HCl, pH 8. In cases indicated, fresh or salt-treated chromatin was sheared by sonication with three 1 min bursts at 6 A. The supernatant from the 100 000 *g* centrifugation containing dissociated proteins was dialysed against 0.1 mM PMSF, freeze-dried and analysed by gel electrophoresis.

2.3. *SDS-Polyacrylamide gel electrophoresis*

Freeze-dried protein sample was dissolved in a buffer containing 1% sodium dodecyl sulphate (SDS) and electrophoresed in 7.5% polyacrylamide gel–1% SDS as described previously [3]. Densitometric tracings were taken at 550 nm in a Joyce Loebl Chromoscan.

2.4. *Measurement of the CD spectrum*

This was recorded at 25°C in a Jasco Model J-20 spectropolarimeter (Japan Spectroscopic Co., Tokyo, Japan), in a 1 cm or 2 cm light-path. Concentration of the chromatin sample in 10 mM Tris·HCl, pH 8, was adjusted to give A_{260} of 1.2–1.5. Spectra were also taken in the presence of 0.5% SDS. Mean residue ellipticity, θ, is expressed in terms of deg × cm^2 × $dmol^{-1}$ on the basis of DNA residue concentration.

2.5. *Assays*

DNA was determined by ultraviolet absorption at 260 nm in 0.5% SDS [7], using an ϵ_P of 6 800 M^{-1} cm^{-1}. It was also determined fluorometrically [8], with calf thymus DNA (type I, Sigma) as standard. RNA was determined by orcinol reaction [9], with yeast RNA (type XI, Sigma) as standard. Protein was determined according to Lowry et al. [10], with bovine serum albumin as standard.

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3. Results

Mass ratio of DNA: protein: RNA was 1:2.1:0.11 for our cerebral chromatin, and 1:2.0:0.13 for liver chromatin. With 0.3 M NaCl, 15–20% of the proteins is dissociated from the unsheared chromatin, which can be recovered in the supernatant after the centrifugation at 100 000 *g* for 2 h. Gel electrophoretic analysis of extracted proteins has shown that none of the histone species is extracted at this salt concentration. The extract from the cerebral chromatin is characterised by a relative wealth of non-histone proteins that migrate slower than histone f1, whereas in the extract of liver chromatin, those migrating faster than f1 predominate (fig.1). Thus the characteristic of the non-histone banding pattern observed in the cerebral and liver chromatins [3] is retained in the respective salt extracts. However, 0.3 M NaCl fails to extract a group of non-histone proteins with the highest mol. wt, which are present in the native cerebral chromatin (group F in [3]).

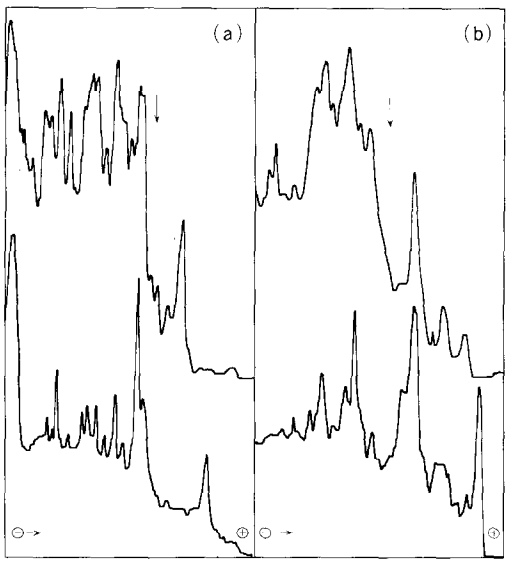

Fig.1. Polyacrylamide gel electrophoresis of non-histone proteins. (a) Proteins from the cerebral chromatin. (b) Proteins from the liver chromatin. Upper tracings: non-histone proteins dissociated from chromatin with 6 M urea–0.4 M guanidine hydrochloride [6], and separated by SP-Sephadex C-25 column chromatography in 6 M urea–0.15 M NaCl [21]. Lower tracings: non-histone proteins extracted from the chromatin with 0.3 M NaCl (see Materials and methods). Vertical arrows indicate the position where histone f1 is to locate (cf. [3]). Horizontal arrows indicate the direction of electrophoretic migration.

As compared with unsheared liver chromatin, unsheared cerebral (neurone-rich) chromatin is characterised by its higher ellipticity at the 270 nm region [1,2]. When unsheared chromatin is extracted with 0.3 M NaCl, ellipticity at 260–300 nm is drastically reduced to the level of 1600 – 1800 deg × cm^2 × $dmol^{-1}$, and the difference between the cerebral and liver chromatin is no longer apparent (fig.2). Upon sonication of salt extracted chromatin, ellipticity is again raised to the level almost identical with that of unsheared chromatin. Under these circumstances, however, the ellipticity at the 270 nm region is preferentially reduced to such an extent that a unique difference between the cerebral and liver chromatins is abolished (fig.2).

When the native chromatin is sonicated, the ellipticity at 260–300 nm is increased by approx. 10%. When the chromatin is first sonicated and then extracted with 0.3 M NaCl, the positive band at 260–300 nm is almost completely lost, and the CD spectrum resembles that of DNA in C form (data not shown; [11,12]). However, when the sonicated-and-extracted chromatin is again sonicated, CD spectrum becomes similar to that of unsheared-extracted-and-sonicated chromatin. It is also noted that a small negative band at 310 nm observed in unsheared chromatin is always lost when sonication is once applied to chromatin at any stage of treatment.

In 0.5% SDS, CD spectrum of the chromatin is identical with that of free DNA in B form (with the maximum ellipticity of 7500–8600 deg × cm^2 × $dmol^{-1}$), even though chromatin has been treated in different ways as stated above.

Extraction of unsheared chromatin with 0.3 M NaCl is accompanied by a reduction of the negative ellipticity at 220–240 nm (fig.2), reflecting the loss of a portion of proteins originally bound to chromatin.

4. Discussion

Sonication, high-speed blending, or other types of shearing has been widely used to reduce the particle size of chromatin, and thereby to facilitate the various

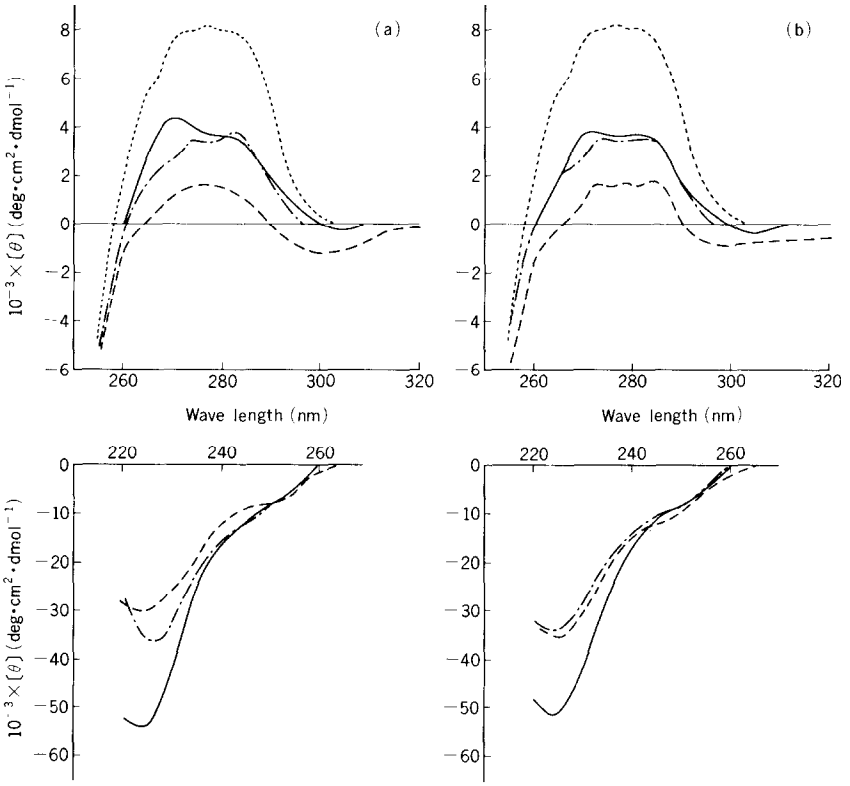

Fig.2. Circular dichroism spectra of chromatin treated in different ways. Spectra were taken with samples dissolved in 10 mM Tris·HCl, pH 8. Native unsheared chromatin (———); after the extraction with 0.3 M NaCl (----); after the extraction with 0.3 M NaCl, and subsequent sonication (-·--); and free DNA (. . . .). (a) Cerebral chromatin. (b) Liver chromatin.

aspects of chromatin analyses. It is generally accepted that shearing does not cause any appreciable dissociation of chromatin proteins. However, evidence has also accumulated for artefactual changes caused by shearing applied during or after the preparation of chromatin, i.e. a reduction in T_m [13], an increase in the ellipticity at 282.5 nm [12], changes in the RNA synthesising activities [14], and a loss of the repeating structure of chromatin [15]. Our present observation adds another evidence for the conformational change of chromatin produced by shearing.

Hjelm and Huang [16] have recently shown with chromatins from chick embryo brain, pig cerebellum and other tissues that treatment of the chromatin with 0.2–0.35 M NaCl produces a rather selective decrease in ellipticity at the 270 nm region. In our present experiment, their finding was confirmed with sonicated chromatin, but not with unsheared chromatin (fig.2).

Lin et al. [17] have reported the virtual absence of labelled RNA and DNA in 0.25 M NaCl extract of chromatin from fibroblast cells that had been incubated with [^{3}H] uridine or [^{3}H] thymidine. In our experiment, RNA extracted from unsheared chromatin with 0.3 M NaCl amounts at maximum to 3% of DNA contained in the starting chromatin, and DNA was not detectable in the extract. Double-stranded RNA, base-paired region of tRNA and DNA-RNA hybrid are known to form a positive peak at 261–267 nm with the maximum ellipticity of approx. 25 000 deg × cm^2 × dmol^{-1} [18–20]. Suppose RNA in chromatin had a similar peak in the CD spectrum, a portion of RNA extracted with 0.3 M NaCl would contribute only 750 deg × cm^2 × dmol^{-1} at the most to the ellipticity at 261–267 nm. The contribution at 270 nm (and at longer wave lenth range) would be even smaller, since the peak declines sharply on both sides [18–20]. After the treatment of chromatin

with 0.3 M NaCl, ellipticity at 270–300 nm is decreased by approx. 2000 deg × cm^2 × $dmol^{-1}$, i.e. to an extent that is not to be accounted for by the loss of RNA alone.

Crossover point of the positive peak exhibited by DNA in B form is at 258 nm, whereas that of double-stranded RNA at wave length range shorter than 250 nm. If RNA contributes significantly to the CD spectrum at around 270 nm, then a blue-shift of the crossover point to shorter than 258 nm would be expected to occur. However, the crossover point of chromatin is never blue-shifted from that of DNA (fig.2). This is so even when a red-shift caused by interaction of proteins with DNA is abolished by 0.5% SDS.

Thus, in agreement with Lin et al. [17], it seems reasonable to conclude that changes in the CD spectrum of chromatin produced by extraction with 0.3 M NaCl are largely attributable to non-histone proteins, even though a smaller role played by RNA [16] may not be ruled out.

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