On the structure of active chromatin

A flow linear dichroism study of chromatin fractionated by nuclease digestion

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Received 27 February 1984

Nuclei from Ehrlich ascites cells were treated with micrococcal nuclease or DNase I and extracted with 1 mM EDTA. The chromatin fraction released by this procedure showed positive flow linear dichroism (LD) at low salt (2 mM NaCl) while the non-released fraction had negative LD. Furthermore, the chromatin structure responsible for the positive LD was found to be labile: The LD was reduced by heat (37°C) or RNase treatment and inverted to a negative LD by electric fields (10 kV/cm) and by the presence of DNA binding dyes.

Active chromatin *Flow linear dichroism* *Nuclease digestion* Chromatin structure

1. INTRODUCTION

The basic chromatin structure is now well established: The 10 nm fiber observed by electron microscopy at low salt (<30 mM) consists of 'beads on a string' nucleosomes [1–3] and at higher salt concentrations (30–100 mM) this coils into the 30 nm solenoid [4–6]. However, the nature of the structural differences that distinguish transcriptionally active chromatin from its inactive state is still in dispute [7]. Electron microscopic studies suggest that transcriptionally active chromatin does not contain nucleosomes [8], while nuclease digestion studies indicate these to be present in expressed genes [9,10].

Electric dichroism, and more recently flow linear dichroism, have proven very powerful tools in the analysis of DNA orientation in chromatin, and these techniques sensitively register the changes in chromatin structure induced by changes in the ionic environment [11–15]. However, a discrepancy concerning results at low ionic strength (<100 nM) has arisen. Using electric dichroism negative values of the reduced dichroism indicating negligible nucleosome tilt (the 'edge to edge' model) have consistently been reported [11–13], while the flow linear dichroism method has usually resulted in a positive reduced dichroism indicating a considerable nucleosome tilt [14,15].

Bearing on these results we now report that part of the chromatin which is released by treatment of nuclei with micrococcal nuclease or DNase I shows a positive flow linear dichroism at low ionic strength (<100 mM) while the non-released chromatin shows a negative flow linear dichroism under the same ionic conditions. Furthermore, the chromatin structure responsible for this positive linear dichroism was found to be labile: The dichroism was significantly reduced by heat (37°C) or RNase treatment and was inverted to a negative dichroism by moderate electric fields (10 kV/cm) or the presence of DNA binding dyes (ethidium bromide or methyl green).

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Published by Elsevier Science Publishers B.V.
00145793/84/$3.00

2. METHODS

2.1. Chromatin preparation

Nuclei were isolated from Ehrlich ascites tumor cells [16]: 6 g of washed cells were lyzed by gentle homogenization in 140 mM NaCl, 10 mM Tris–HCl, 1.5 mM $MgCl_2$, 0.5% Nonidet P40 (pH 7.4) and isolated by pelleting (3500 × g, 20 min) through a sucrose cushion [10 mM Tris–HCl, 5 mM $MgCl_2$ 1% Nonidet P40, 0.8 M sucrose (pH 7.4)]. The nuclei were resuspended in 10 ml of 0.34 M sucrose, 60 mM KCl, 1.5 mM NaCl 1 mM $CaCl_2$, 0.5% Nonidet P40, 10 mM Tris–HCl (pH 7.4) and incubated with nuclease (200 units micrococcal nuclease or 20 μg DNase I (Boehringer Mannheim)) for 5 min at 37°C. The nuclei were isolated by centrifugation (2000 × g, 5 min) and resuspended in 10 ml of 1 mM EDTA. Centrifugation at 10000 × g for 10 min gave the supernatant fraction and the pellet fraction which were allowed to swell in 10 ml of 1 mM EDTA. A size distribution of the DNA from a typical preparation is apparent from the agarose gel analysis shown in fig.1.

Chromatin from rat liver was prepared as in [17].

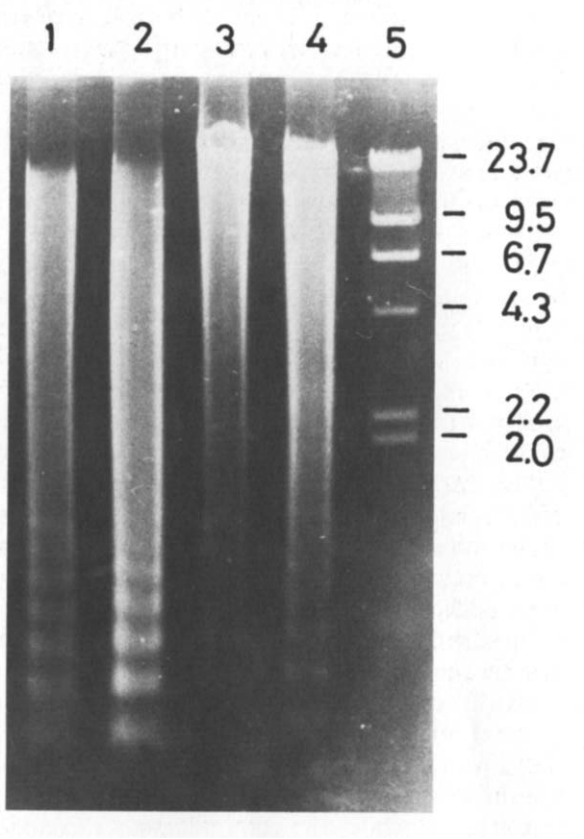

Fig.1. Gel electrophoretic analysis of the DNA from chromatin samples used for flow linear dichroism measurements. The DNA was isolated by phenol extraction and electrophoresed in 1% agarose in 40 mM Tris–HCl, 5 mM NaOAc, 2 mM EDTA (pH 8.0). Lanes 1 and 2: supernatant fraction of chromatin from nuclei treated with 20 and 60 units/ml micrococcal nuclease respectively. Lanes 3 and 4: pellet fractions corresponding to 1 and 2. Lane 5: DNA-size marker (in kbp): λ-DNA restricted with *Hind* III. Similar DNA distributions were obtained for nuclei treated with 4 μg/ml DNase I.

2.2. Linear dichroism measurements

Linear dichroism, $LD = A_{\parallel} - A_{\perp}$, was measured by using a Jasco J-500 spectropolarimeter converted to linear mode as in [18], on the chromatin solutions flow-oriented in a Wada-Kozawa Couette cell [19] as in [14,20]. The reduced flow linear dichroism, $LD_r = LD/A_{iso}$ was calculated with A_{iso}, the isotropic absorbance, measured on a Cary 219 spectrophotometer which was wave-

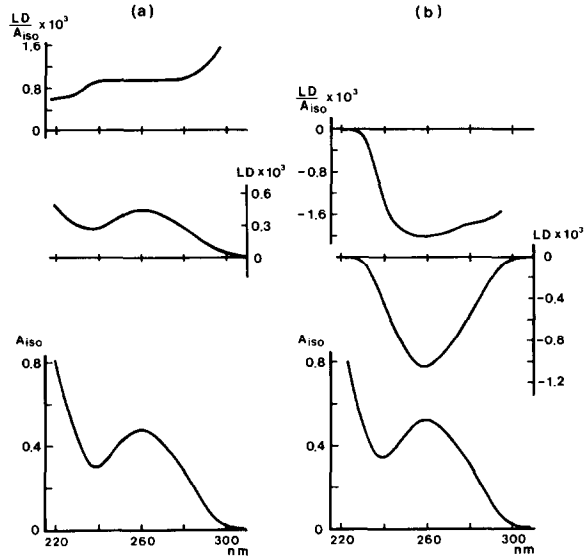

Fig.2. Linear dichroism (*LD*), isotropic absorbance (A_{iso}), and reduced dichroism (LD/A_{iso}) curves of supernatant (a) and pellet (b) fractions of mouse ascites chromatin in 1 mM NaCl and 0.2 mM EDTA. *LD* was measured at flow-gradient (*G*) 1800 s^{-1} and 8°C. Optical path length 1 mm for both *LD* and A_{iso} measurements. The samples were prepared by brief digestion with micrococcal nuclease (practically identical spectra were obtained with DNase I).

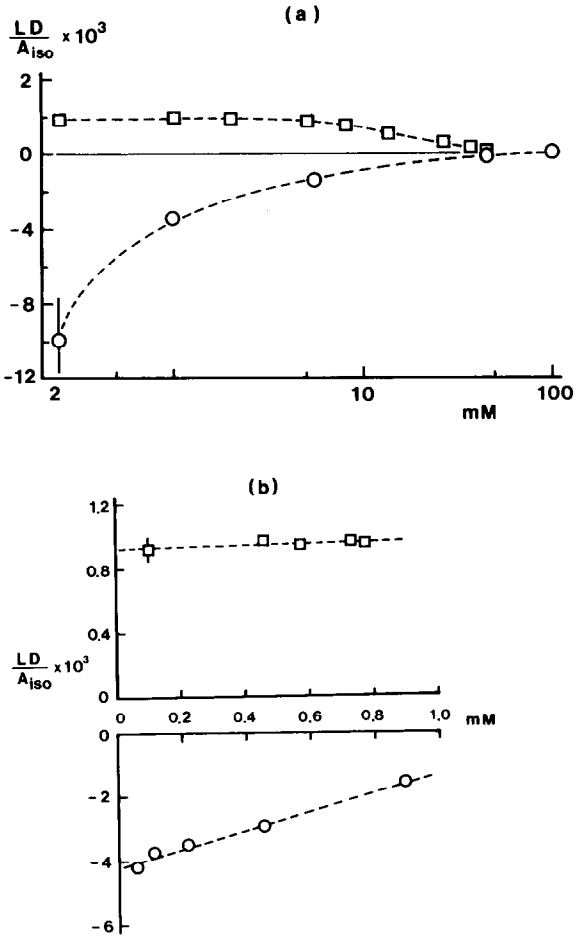

Fig.3. (a) Reduced dichroism (LD/A_{iso}) at 260 nm of supernatant (□) and pellet (○) chromatin at different salt concentrations (including 0.2 mM EDTA). LD measured at $G = 1800\,s^{-1}$ and 8°C on chromatin of a concentration of approx. 0.1 mM DNA phosphate. (b) Reduced dichroism at 260 nm of supernatant (□) and pellet (○) chromatin at various chromatin concentrations. LD measured at $G = 1800\,s^{-1}$ and 8°C in 1 mM NaCl and 0.2 mM EDTA.

length-matched with the Jasco spectropolarimeter. (For the wavelength variation of LD_r or DNA and its structural interpretation, see [22].) All measurements refer to 8°C.

3. RESULTS

The results of flow linear dichroism measurements on the nuclease-sensitive chromatin fraction (supernatant) and the nuclease-resistant fraction (the swollen pellet) at 2 mM NaCl are shown in fig.2. It is observed that while the pellet fraction exhibits a negative LD (and thus also negative LD_r) the supernatant clearly shows positive LD (and LD_r) over the range 220–300 nm.

The dependency of the LD_r of both these fractions on salt concentration and chromatin concentration is shown in fig.3. The LD_r of both fractions approaches zero at approx. 50 mM NaCl. At this ionic strength it has been shown that chromatin exists in a dense form: the 30 nm solenoid fiber [4–6].

The LD_r of the supernatant did not reveal any significant change with decreasing chromatin concentration indicating that no aggregation was taking place, whereas that of the pellet fraction changed by more than a factor of 2.

The lability of the chromatin structure in the supernatant fraction responsible for the positive

Table 1

Reduced linear dichroism (LD_r) of chromatin fractions

Chromatin	Treatment	LD_r(260 nm)($\times 10^3$)
Pellet	none	−3.5
Supernatant	none	+0.9
	37°C (30 min)	+0.1
	RNase (37°C, 30 min, 5 mM NaCl)	+0.65
	10 kV/cm	large negative[a]
	ethidium bromide ($r = 0.05$)	−2.7
	methyl green ($r = 0.05$)	−2.6

[a] Measured as electric dichroism

Flow dichroism results at $G = 1800\,s^{-1}$ on samples in 1 mM NaCl and 0.2 mM Na_2EDTA

LD_r was demonstrated in various ways (table 1). Upon heating to 37°C for 30 min the LD_r was reduced by 90% and treatment with RNase similarly caused a 30% decrease in the LD_r. The application of a moderate electrical field (10 kV/cm) which is routinely used in electric dichroism measurements [5,6,11–13] caused an immediate inversion of the LD_r. The presence of DNA-binding dyes has a similar effect.

4. DISCUSSION

Our data clearly demonstrate the existence of a structural difference, detectable by flow *LD*, between the chromatin fraction which is readily released from nuclei upon brief digestion with nucleases (micrococcal or DNase I) and the chromatin fraction which is not.

Furthermore, the discrepancy between *LD* measurements on solubilized chromatin performed by flow orientation, showing positive LD_r for chromatin prepared under mild conditions [14,15], as opposed to electric field orientation giving always negative *LD* [11–13], may be explained by our observation that LD_r is inverted to a negative value under the influence of a moderate electric field (table 1).

Although it is premature to propose a model for the chromatin structure of the supernatant fraction and evaluate the biological significance of the results presented here, a number of points should be made.

(i) A positive LD_r for chromatin shows that the bases of the DNA helix are strongly tilted relative to the orientation axis, which tentatively indicates a heavily supercoiled DNA [14,20–23]. This interpretation is consistent with the observation that the addition of ethidium bromide, which is known to unwind DNA [24], causes an inversion to negative LD_r, i.e., the DNA adopts a less supercoiled conformation.

(ii) The structure responsible for the positive LD_r is quite labile and may also to some extent be dependent on the presence of RNA (table 1).

(iii) It has been established that the chromatin fractions which are released by nucleases are enriched in transcriptionally active DNA regions [7,25,26]. Thus the intriguing possibility exists that the differences in chromatin structure observed by linear dichroism are related to the functional states of chromatin.

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