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FLOW LINEAR DICHROISM SUPPORTS AN ACCORDION MODEL FOR THE SALT-INDUCED CONDENSATION OF CHROMATIN

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Introduction

It is generally accepted that the major part of DNA in eucaryotic cells is packed into a nucleosomal chromatin fibre structure with a diameter of approximately 30 nm [1]. *In vitro*, chromatin adopts at low ionic strength an extended structure with ~10 nm in diameter [2], which upon addition of cations such as Na^+, Mg^{2+} or spermine condenses into a 30 nm fibre [3]. In this report we study the condensation of chromatin at varying ionic strength, by measuring the linear dichroism (LD) of the absorption of the DNA bases and of an intercalative dye, methylene blue (MB), which selectively binds to the linker DNA regions.

Methods

Chromatin preparation. Micrococcus nuclease solubilized chromatin was isolated from nuclei of Ehrlich ascites tumor cells as previously described [4].

Optical measurements. LD, defined as the differential absorption of polarized light with a direction parallel and perpendicular, respectively, to a macroscopic direction of orientation. LD was measured in a Jasco J-500 spectropolarimeter as described elsewhere [5]. The chromatin samples were oriented in a Couette cell with an inner rotating cylinder [6]. The cell is manufactured of high-quality UV-transparent and birefringence-free fused silica. The LD was calibrated to be absolutely correct within 3 % (absorbance units) at all wavelengths [7]. The applied flow-gradient was 1800 s^{-1}. Control experiments at lower gradients, where the orientation was increased by addition of sucrose, in no case revealed a change of signs, confirming that the applied shear does not significantly perturb the chromatin structure.

Reduced linear dichroism, $LD^r = LD/A_{iso}$, was calculated with A_{iso}, the isotropic absorbance, measured on a CARY 219 spectrophotometer. All measurements were made at 8^0 C. Methylene blue was standard reagent of p.a. quality.

Results and Discussion

LD^r depends on the local orientations of the light-absorbing chromophores relative to the orientation axis of chromatin and also on the hydrodynamic orientation properties of the chromatin fibre in the flow field. The prominent transition moments of both DNA bases and MB are restricted to the molecular planes of these chromophores, and the chromatin particle is, by virtue of hydrodynamic arguments, oriented with its fibre axis parallel to the flow. Therefore, a negative and a positive LD^r will be observed if the chromophores are oriented preferentially perpendicular and parallel, respectively, to the fibre axis of chromatin. MB, at low binding ratios, is known to intercalate selectively in the linker regions of chromatin and can thus be used to probe the linker orientation [4].

In Fig. 1a the LD^r of the DNA base (260 nm) and MB (674 nm) absorption is shown as a function of Na^+ concentration, and in Fig 1b the LD^r of the DNA bases is shown as a function of Na^+ and Mg^{2+} concentration. It is seen that at low ionic strength the LD^r signals of both the MB and the DNA base absorption are strongly negative. This indicates a well-oriented structure where the linkers and the chromatosomes, with their flat faces, are oriented preferentially parallel with the chromatin fibre axis. The LD^r of the DNA bases becomes positive at approximately 2.5 mM Na^+ (or ~0.07 mM Mg^{2+}) whereas the LD^r of MB changes signs at somewhat higher salt concentration. The change of signs of the LD^r signals indicates a tilt of

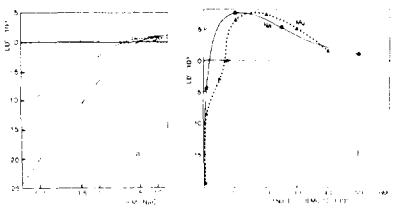

Fig. 1. *Dependence of linear dichroism on ionic strength.* **a**: LD^r of DNA bases at 260 nm (- - -) and of MB at 674 nm (———) as a function of Na^+ concentration. **b**: LD^r of DNA bases at 260 nm as a function of Na^+ (———) and Mg^{2+} (●●●●●) concentration. Note the different concentration scales. Samples contain 15.2 mM DNA in phosphate, 0.05 mM Na_2 cacodylate and 0.25 mM EDTA. Binding ratio of MB 0.005. Flow gradient 1800 s^{-1}. Fig. a is reproduced by permission from Biochemistry 1985, **24**, 6336.

both linkers and chromatosomes to an orientation preferentially perpendicular to the chromatin fibre. The non-monotonic behavior of the LD^r signal, reaching a positive maximum at an intermediate ionic strength, before it gradually decreases and eventually vanishes (Fig. 1b), implies that the transition must involve, in addition to an initial extended fibre (negative LD^r) and a final fully condensed fibre (vanishing LD^r), also at least one intermediate structure.

Based on these results, we propose that the effect of increasing ionic strength is a *continuous* change of linker and chromatosome arrangement towards a condensed structure. Already at approximately 2.5 mM Na^+ (or ~0.07 mM Mg^{2+}) the preferential orientation of the linker and chromatosomal faces becomes perpendicular to the fibre. Electrostatic repulsion between DNA strands in adjacent nucleosomes makes the fibre remain essentially extended. Further higher ionic strength leads to shielding of the DNA phosphates, and enables face-to-face contact between the chromatosomes, and the fibre condenses. The condensed structure has poor orientation properties and consequently low LD^r.

A model for the salt-induced folding of chromatin that would be consistent with these data, is shown in Fig 2. The condensation, upon increasing salt, is achieved by a *continuously* increasing inclination of both linkers and nucleosomes together with a compression of the fibre (like of an accordion). At ultimate chromatin condensation, according to this proposal, the nucleosomes are arranged in a pentagonal structure, every chromatosome facing its sixth neighbor. Such an arrangement is supported by the

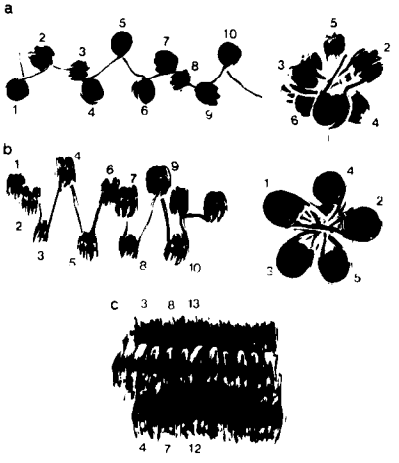

Fig. 2. *Proposed Accordion Folding Model of chromatin.* Extended form in ~1 mM NaCl (a), intermediate form in ~10 mM NaCl (b) and condensed form in ~50 mM NaCl (c).

observation of an increased stability of 30 nm fibres containing more than 5 nucleosomes [8].

Summary

The salt-induced condensation of chromatin has been studied with flow-linear dichroism technique using an intercalative dye (methylene blue) to selectively monitor the linker orientation. At low ionic strength both linkers and chromatosomes (with their flat faces) are oriented preferentially parallel to the chromatin fibre axis. With increasing ionic strength both linkers and chromatosomes tilt sucessively towards a perpendicular orientation.

Based on these results and structural considerations, an '*Accordion model*' with a pentagonal nucleosomal arrangement is proposed for the salt-induced condensation of chromatin.

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