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Higher order folding of chromatin is induced in different ways by monovalent and by bivalent cations

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The condensation of the 10 nm chromatin filament in the 30 nm fiber by monovalent cations, polyamines and bivalent cations was studied with light scattering at 90° and flow linear dichroism methods. It was found that monovalent cation- and polyamine-induced folding was a two-step process: a precondensation, when a rotation of nucleosomes takes place only, and a condensation step without changes in nucleosome orientation. Divalent cations affected the structure of chromatin in one step only – condensation of the chromatin filament being accompanied by nucleosome reorientation.

Chromatin structure; Light scattering; Flow linear dichroism

1. INTRODUCTION

The conformational transitions of chromatin have been the subject of intensive studies in connection with the structure of the genetic apparatus of eukaryotes. A substantial contribution in this respect was made by the use of some physical methods [1–6]. Here, the effect of cations and polyamines on the condensation of chromatin was followed by the changes in light scattering and flow linear dichroism.

2. MATERIALS AND METHODS

Calf thymus nuclei were isolated by the procedure of Blobel and Potter [7]. Chromatin was prepared as described in [6] and used after dialysis against 0.1 mM EDTA, pH 7.0. The integrity of the proteins was checked electrophoretically [8].

Flow linear dichroism (LD) measurements were carried out as in [6]. The variations in intensity of the scattered light $I(90°)$ were measured at 90° to the monochromatic beam (λ = 350 nm) using an Aminco SPF 1000 spectrofluorimeter. Optical measurements were made by adding appropriate amounts of the respective cations directly to the cuvette.

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3. RESULTS AND DISCUSSION

3.1. *Flow linear dichroism*

The dependence of the reduced LD at 258 nm of chromatin on the concentration of monovalent cations (Na^+, K^+), divalent cations (Ca^{2+}, Mg^{2+}) and polyamines (spermine, spermidine) is presented in panels B of figs 1–3, respectively. At 0.1 mM EDTA chromatin exhibits a high negative LD value (see also [6]). Titration of chromatin with increasing concentrations of the cations resulted in drastic and differential changes in LD, depending on the cations used. Monovalent cations and polyamines showed similar effects: a several-fold decrease in amplitude of the negative signal with a subsequent change in the sign to positive at concentrations of 2 mM, 4 μM and 9 μM for Na^+ (K^+), spermidine and spermine,

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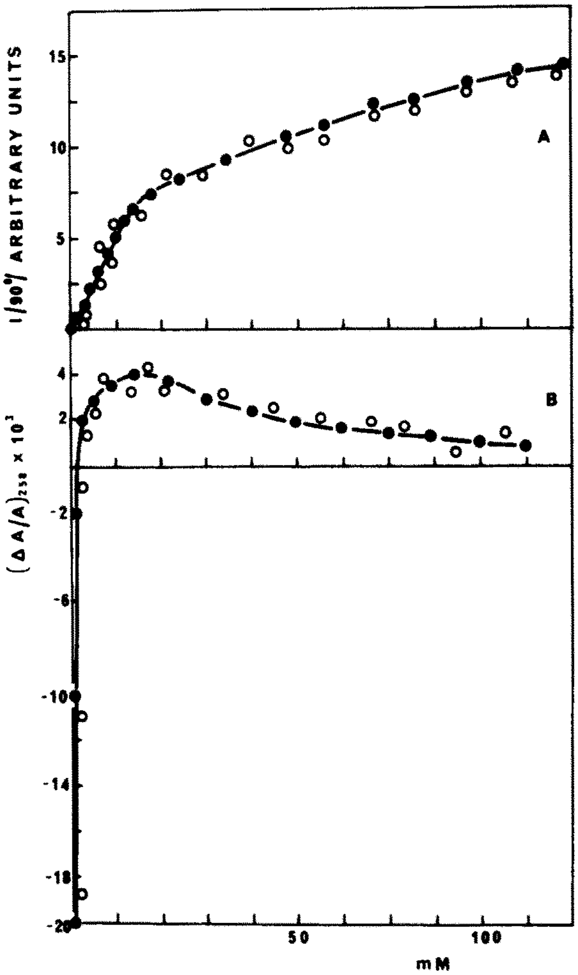

Fig.1. Dependences of the reduced linear dichroism $(\Delta A/A)_{258}$ (A) and the intensity of the scattered light at 90°, I(90°) (B), of chromatin on the concentration of NaCl (●—●) and KCl (○—○).

respectively. Further increase of the cation concentration led to an increase in amplitude of the signal, followed by a gradual decrease of LD which preserved the positive sign in the range of concentrations used.

The effect of the divalent cations was found to differ strongly. The negative LD signal decreased several-fold but no change in the sign of LD was observed up to 0.2 mM Ca^{2+} (Mg^{2+}), when the LD value dropped to zero.

3.2. *Light scattering*

The cation-induced condensation of chromatin

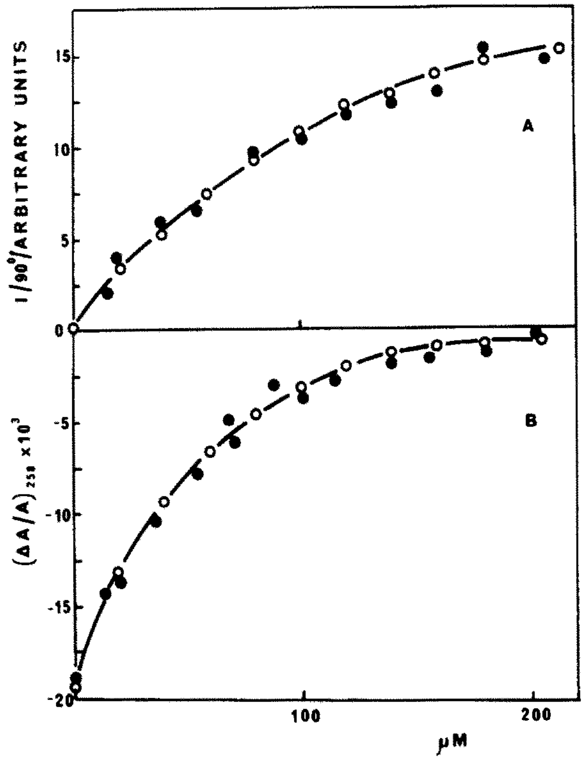

Fig.2. Dependences of the reduced linear dichroism $(\Delta A/A)_{258}$ (A) and the intensity of the scattered light at 90°, I(90°) (B), of chromatin on the concentration of $MgCl_2$ (●—●) and $CaCl_2$ (○—○). The data are obtained at a chromatin concentration E_{260} = 1.0.

was followed by light scattering at 90°, the results being presented in panels A of figs 1–3. The intensity of the scattered light I(90°) increased to a plateau, which was reached at about 100 mM NaCl (KCl), 0.2 mM $CaCl_2$ ($MgCl_2$), 16 μM spermine and 24 μM spermidine. Over these concentrations I(90°) sharply increased, due to aggregation. In the case of polyamines, a sigmoidal curve was observed, suggesting a co-operative binding of polyamines to chromatin.

LD reflects both the orientation of the flat faces of nucleosomes relative to the fiber axis and the extent of compactness of the chromatin fiber [6], while the changes in intensity of the scattered light at 90° are a measure of the condensation only [9,10]. The combination of the two methods enabled us to discriminate the effects of the cations on the reorientation of nucleosomes from those on the condensation of chromatin.

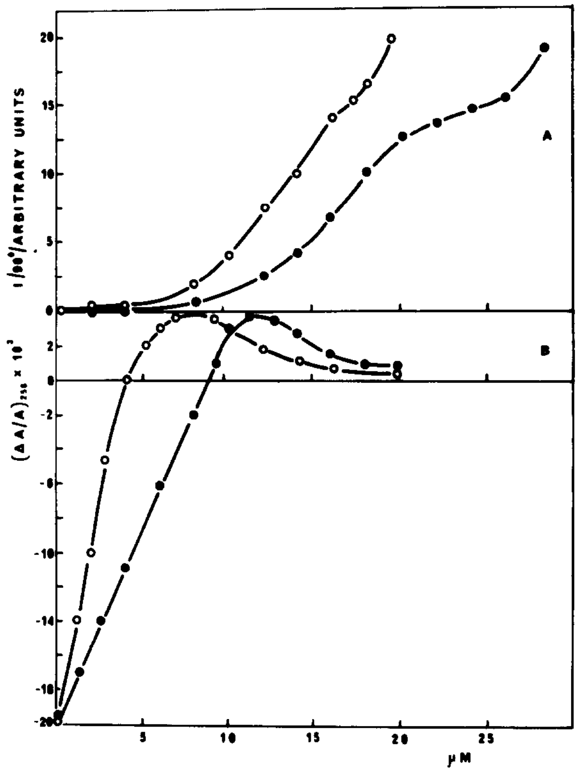

Fig.3. Dependences of the reduced linear dichroism $(\Delta A/A)_{258}$ (A) and the intensity of the scattered light at 90°, $I(90°)$ (B), of chromatin on the concentration of spermidine (●—●) and spermine (○—○). The concentration of the chromatin samples used was E_{260} = 1.0.

Monovalent cations and polyamines affected the chromatin structure in a similar way, as judged by the LD and light scattering data (figs 1,2). The effect of spermidine was observed at a higher concentration than that of spermine, due to the lower binding constant of spermidine (Makarov, V. et al., unpublished). The change in the sign of LD reflected a reorientation of the nucleosome discs relative to the fiber axis [4,6], which are predominantly parallel to the fiber axis at 0.1 mM EDTA. Increase in the concentration of monovalent cations and polyamines caused a rotation of nucleosomes towards an orientation of about 40° to the fiber axis, reached at about 2 mM NaCl (KCl), 4 μM spermine and 9 μM spermidine. When the changes in LD were compared to those of light scattering it became clear that the concentration at which the sign of LD was changed to positive only negligibly affected the intensity of the scattered light: the increase observed did not exceed 5% of the total effect. This is interpreted to mean that changes in LD, including its sign, are due to a rotation of the nucleosomes only. Further increase in the cation concentration resulted in a sharp increase in $I(90°)$ which reached a plateau before the aggregation occurred (not shown). The changes in positive LD reflect mainly the condensation process, since the rotation of nucleosomes does not exceed 3° [6]. It follows, therefore, that the condensation of chromatin in the presence of monovalent cations and polyamines is not accompanied by nucleosome rotation. In other words, the effect of monovalent cations and polyamines on the chromatin structure is a two-step process: a precondensation, when a rotation of nucleosomes takes place only, and a condensation step, when the nucleofilament is folded in the 30 nm fiber without changes in nucleosome orientation. Such a condensation might be accomplished in an 'accordion' like manner [4,11]. Unlike monovalent cations and polyamines, divalent cations induced the condensation in one step: LD and $I(90°)$ changed in parallel. The value of the reduced LD upon folding of the fiber dropped to zero but did not change the sign to positive. For these reasons one cannot differentiate to what extent the decrease in amplitude of the negative signal reflects the reorientation or formation of irregular structures with a low LD value. Assuming a regular structure of the folded fiber the faces of the nucleosomes should be tilted about 36° (the magic angle) from the axis, i.e. divalent cation-induced condensation of chromatin proceeds with a reorientation of nucleosomes from parallel to a position inclined to the fiber axis. Another explanation suggests that the divalent cations induce condensations along the fiber with formation of structures exhibiting an LD signal close to zero. The increase in cation concentration enhances the number of these structures, thus leading to the final folded structures with LD close to zero.

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