Interaction and conformational changes of chromatin with divalent ions

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ABSTRACT

We have investigated the interaction of divalent ions with chromatin towards a closer understanding of the role of metal ions in the cell nucleus. The first row transition metal ion chlorides MnCl₂, CoCl₂, NiCl₂ and CuCl₂ lead to precipitation of chicken erythrocyte chromatin at a significantly lower concentration than the alkali earth metal chlorides MgCl₂, CaCl₂ and BaCl₂. A similar distinction can be made for the compaction of chromatin to the '30 nm' solenoid higher order structure which occurs at lower MeCl₂ concentration in the first group but at the same MeCl₂ concentration within each group. In other experiments in which mixed solutions of NaCl and of MgCl₂ were examined, it is shown that increasing NaCl concentration leads to increasing solubility in the presence of MgCl₂. Best compaction of chromatin was obtained at 40 mM NaCl and 0.8 mM MgCl₂ at a value A₂₆₀ approximately 0.8. Similar experiments were undertaken with mixtures of NaCl and MnCl₂.

INTRODUCTION

The exact role of metal ions in the cell nucleus is difficult to ascertain though the presence of a number of ions, some in larger and some in trace amounts, has been extensively documented.¹⁻³ They are believed to play a role both in catalysis and in structural interplay and stabilization.

Complex problems in cell biology can be approached analytically by probing by various means into composition, structure and function of intact or fragmented cells. On the other hand, it is possible, by a synthetic approach, to probe into the behavior of well defined single cell components, to fit them together Lego-fashion into well defined simple adducts, to study these by exhausting a gamut of experimental conditions, to use the knowledge gained to generate increasingly complex situations, thereby disclosing basic information concerning the operation of the elusive whole. If both approaches converge, significant strides can be made. The second path has been chosen in the present work.

Complexes of DNA with ions and other ligands have been studied in detail.¹⁻³ Distinction could be made between monovalent alkali and divalent
alkali earth ions binding to the DNA phosphate group with varying degree of strength and transition metal ions, interacting with both phosphate groups and bases, affecting DNA conformational and stability properties. Newer approaches, using nuclear magnetic resonance in various forms, may lead to a more detailed understanding of DNA metal ion interactions.4

Far less attention has been devoted to the effect of metal ions on functional and structural aspects of the next higher level of genetic organization, chromatin. Lewis and Laemmli5 believe that one level of DNA organization in metaphase chromosomes is brought about by a scaffolding structure stabilized by metalloprotein interactions. Chromosomes isolated in a metal-depleted form, which generate slow-sedimenting, histone-depleted structures, can be specifically and reversibly stabilized by small amounts of Cu\(^{2+}\), but not by Mn\(^{2+}\), Co\(^{2+}\), Zn\(^{2+}\) or Hg\(^{2+}\); the effect of Ca\(^{2+}\) is less specific than that of Cu\(^{2+}\).

At low ionic strength intact chromatin fragments, composed of DNA, the inner histones and the linker histone H1 (as well as H5 histone in the case of chicken erythrocyte chromatin), behave as extended linear structures. This so-called "10 nm" fiber has been shown to fold into a higher order "30 nm" solenoid6 upon addition of NaCl to approximately 75 mM or MgCl\(_2\) to the much lower concentration of about 0.3 mM. It has been demonstrated that this "30 nm" higher order structure belongs to a hierarchy of native chromosomal structures.7 At this stage it has not been possible to distinguish between the structure obtained by the addition of NaCl or that obtained by the presence of MgCl\(_2\), at concentrations much lower than expected from electrostatic considerations, in relation to the effect caused by monovalent ions. In terms of gross dimensions both structures are indistinguishable. From detailed analysis of light scattering data8 we could show that the "10 nm" structure is not rigid, but coils in solution. A regular rigidly compacted "30 nm" structure consisting of 5.7±0.1 (approximately six) nucleosomes per helical turn of the solenoid is consistent with results from total intensity elastic light scattering, yet detailed analyses of quasielastic light scattering and sedimentation,8 as well as small angle X-ray scattering,9 raise the possibility of alternate models.

The purpose of chromosomal organization is twofold: (a) it is a means of packaging huge linear stretches of genetic material, DNA, in compact form and (b) it must at the same time provide for making DNA available at short notice, for biological function, recognition by and interaction with a host of proteins, leading to DNA replication and transcription in the process of gene expression. Subtle shifts in levels of structure, leading to either tighte-
ning and compactization or to a partial unfolding can be controlled in a variety of ways: by attachment or release of associated proteins, by post-synthetic modifications, or by changes in the availability of specifically interacting ions. Very little is known about the role of divalent ions of various kinds in their interaction with chromatin. Following methodology developed in our recent work on the interaction of chromatin with NaCl and with MgCl₂,⁸ we saw it appropriate to extend these investigations to the study of various divalent ions which may play a role in regulating cellular structure.

MATERIALS AND METHODS

Experimental details mentioned in this section have been described in detail.⁸ Chromatin fragments were obtained from mature chicken erythrocyte nuclei by short digestion with micrococal nuclease in 100 mM KCl, 50 mM Tris-Cl, 1 mM CaCl₂ (pH 8.0), stopped by addition of EDTA to 5 mM, pelleting of the nuclei, release of chromatin by lysis of the pellet by dialysis against 0.25 mM EDTA. The chromatin was fractionated in a sucrose gradient in 25 mM NaCl, 5 mM Tris-HCl, pH 8.0, 0.1 mM EDTA. Conformational transitions and incipient precipitation as a function of MeCl₂ concentration (in 1 mM Tris-HCl, pH 8) were then followed by determining by laser light scattering, an apparent diffusion coefficient D(90) and the scattered light intensity I(90) at a scattering angle θ = 90°. Experiments were performed at 20°C, A₂₆₀ = 0.8, in 100 μl samples in optically clear cellulose nitrate tubes of the Beckman Airfuge air-driven ultracentrifuge. Prior to light scattering measurements soluble chromatin samples, in 1 mM Tris-Cl, pH 8, were clarified by centrifugation in the Airfuge at 20,000 rpm for 10 min. This corresponds to about 12,000 g. Optically clear concentrated MeCl₂ solutions were added with mixing to yield desired MeCl₂ concentrations. The weight average number of nucleosomes per chromatin molecule, Nw, was estimated from the values of D(90) in 75 mM NaCl, 1 mM Tris-Cl, 0.1 mM EDTA, pH 8.0 (cf, Fig.10, Ref.8).

D(90) is not the true translation diffusion coefficient (which, for particles this size should be obtained by extrapolation to θ → 0) and neither can I(90) be simply related to the scattering I(0) obtained by a similar extrapolation. We are here investigating scattering at right angles from small experimental samples because of the convenience of the procedure and high sensitivity at low concentrations, allowing us to monitor phenomena related to conformational transitions with small amounts of valuable materials. Detailed light scattering experiments in the study of the interaction of chromatin with NaCl...
Figure 1: Apparent diffusion coefficients $D(90)$, $(\square)$, and scattered intensity of light $I(90)$, in arbitrary units, $(\bullet)$, of chromatin fraction ($N_w = 45$), against concentration of divalent electrolytes, $MeCl_2$, at 20°C, $A_{260} \sim 0.8$, (a) alkali earth chlorides; (b) transition metal chlorides. Solutions also contained 1 mM Tris-HCl, pH 8.0.

and $MgCl_2$, including extrapolation to $e \rightarrow 0$, have been reported elsewhere. 8

Chromatin solubility was also determined by adjusting $MeCl_2$ concentration to a given value in chromatin at $A_{260} = 0.8$ in 1 mM Tris-HCl, pH 8.0, allowing the solutes to stand at 4°C overnight. This was followed by centrifugation at 12,000 g for 10 minutes to remove precipitated material, and determination of the supernatant absorption $A_{260}$.

RESULTS AND DISCUSSION

In Fig. 1a is given the scattering behavior of chromatin ($N_w = 45$) in 1 mM Tris-HCl, pH 8.0, with increasing concentration of the alkaline earth metal chlorides $MgCl_2$, $CaCl_2$ and $BaCl_2$. $D(90)$ increases from about $4.9 \times 10^{-8}$ cm$^2$/sec in the absence of $MeCl_2$ to a maximum value of $8.2 \times 10^{-8}$ cm$^2$/sec at about 0.4 mM of the $MeCl_2$. The behavior is rather similar for all three salts and indicates compaction with increasing salt concentration from the "10 nm" fiber to the "30 nm" solenoid. Further increase in divalent salt concentrations leads to a decrease in $D(90)$ following aggregation and incipient pre-
Figure 2a: Chromatin solubility at 4°C against concentration of divalent electrolytes; (■) MgCl\textsubscript{2}, (▲) CaCl\textsubscript{2}, (▲) BaCl\textsubscript{2}, (●) MnCl\textsubscript{2}, (○) CoCl\textsubscript{2}, (□) NiCl\textsubscript{2}, (+) CuCl\textsubscript{2}; N\textsubscript{w} = 45, A\textsubscript{260} ~ 0.8. Solutions also contained 1 mM Tris-HCl, pH 8.0.

Figure 2b: Electrophoretic analysis of histones in 15% polyacrylamide gels; A, typical chromatin fraction used in this work, stored in 1 mM Tris-HCl, pH 8.0, clarified for light scattering at 12,000 g for 10 minutes. B, supernatant (containing 99% of chromatin fraction) after centrifugation at 12,000 g for 10 minutes to remove precipitated chromatin, following adjustment of MgCl\textsubscript{2} to 0.5 mM and storage overnight at 4°C; C, same as B, containing 70% of chromatin fraction, MgCl\textsubscript{2} adjusted to 0.6 mM; D same as B containing 42% of chromatin fraction MgCl\textsubscript{2} adjusted to 0.7 mM.

Cipitation of chromatin molecules. These observations are paralleled in the behavior of I(90) which first increases moderately because of reduced destructive scattering interference (at right angle scattering) following chromatin compaction, and then increases drastically beyond 0.4 M MeCl\textsubscript{2}, following increase in molar mass due to aggregation.

In Fig. 1b we show, in a similar experiment as described in Fig. 1a, the behavior of the transition metal salts MnCl\textsubscript{2}, CoCl\textsubscript{2} and CuCl\textsubscript{2} which again form a distinct group, with maximum chromatin compaction now occurring at much lower concentration of salts: 0.2 mM for MnCl\textsubscript{2} and 0.1 for CoCl\textsubscript{2} and CuCl\textsubscript{2}. The compaction is slightly smaller, the maximum D(90) being about 7.4x10\textsuperscript{-6}cm\textsuperscript{2}/sec.

In Fig. 2a we show by solubility determinations, as described above, a similar pattern of behavior characteristic of the two groups of ions (also including NiCl\textsubscript{2} in the transition metal series). In this, somewhat less sensitive procedure, the transition is shifted to slightly higher divalent salt concentrations. In Fig. 2b we show that, in the case of MgCl\textsubscript{2}, chromatin remaining in solution retains a full complement of linker and core histones.
Figure 3: Apparent diffusion coefficients $D(90)$ of chromatin fraction ($N_w = 48$), against MgCl$_2$ concentration, at various concentrations of NaCl: $t=20^\circ C$, $A_{260} \approx 0.8$; (I) zero NaCl, (■) 5 mM NaCl; (○) 8 mM NaCl, (●) 20 mM NaCl, (■) 40 mM NaCl. Solutions also contained 1 mM Tris-HCl, pH 8.0.

This is true even at 0.8 mM MgCl$_2$ (not shown) with only 3% chromatin remaining in the supernatant. Chromatin solubility in CuCl$_2$, at concentrations above 0.3 mM of divalent salt, levels off at 30% solubility, in distinction to the other salts which lead to complete precipitation. The behavior in the presence of CuCl$_2$ has been checked a number of times with different chromatin samples. Copper is known to show strong preference for DNA bases rather than phosphate groups and leads to strong decrease of DNA melting temperatures even at low metal to phosphate ratios. The role of copper in stabilizing the scaffolding

Figure 4: Apparent diffusion coefficients $D(90)$ of chromatin fraction ($N_w = 33$), against MnCl$_2$ concentration, at various concentrations of NaCl. Symbols and experimental conditions as in Fig. 3.
structure in metaphase chromosomes has been indicated.\textsuperscript{5}

In Fig. 3 we show, for a chromatin fraction of \( N_w = 48 \) and in the presence of 1 mM Tris-HCl, pH 8.0, that the stability of chromatin in solutions of MgCl\(_2\) can be shifted to higher MgCl\(_2\) concentrations if increasing concentrations (up to 40 mM) of NaCl are provided. Here the initial \( D(90) \), at zero added MgCl\(_2\), equals \( 4.3 \times 10^{-8} \) cm\(^2\)/sec increasing to \( 6.3 \times 10^{-8} \) cm\(^2\)/sec at 40 mM NaCl (no MgCl\(_2\)) to a maximum of \( 6.8 \times 10^{-8} \) cm\(^2\)/sec at 70 mM NaCl (no MgCl\(_2\)); decrease of \( D(90) \), indicating aggregation, begins at 80 mM NaCl (no MgCl\(_2\)).

Figure 5: Chromatin solubility at \( 4^\circ C \) against concentration of MgCl\(_2\) at various NaCl concentrations. Symbols and experimental conditions as in Fig. 3.

Figure 6: Concentration \( C_{\text{MgCl}_2}^{1/2} \) (at midpoint (50%) of chromatin precipitation) of MgCl\(_2\) (■) and MnCl\(_2\) (○), against NaCl concentration. Data taken from Fig. 5.
The maximum value of D(90) at 40 mM NaCl, achieved at 0.8 mM MgCl$_2$ is $7.9 \times 10^{-8}$ cm$^2$/sec. These are the best conditions for compaction obtained and may in fact correspond to the best organized higher order structure achievable in solution. Similar conclusions can be derived from data shown in Fig. 4 for the scattering properties of a chromatin fraction (N$_w$ = 33) in mixtures of NaCl and MnCl$_2$.

Chromatin solubility has also been determined at 4°C (by the methods described above) in mixtures of NaCl and, on the one hand MgCl$_2$, and MnCl$_2$ on the other (Fig. 5). The data are summarized in Fig. 6. The basic conclusions emerging from the scattering measurements (Figs. 3 and 4) are similar to those derived from the precipitation study (Figs. 5 and 6), though it clearly emerges that the scattering experiments sense phenomena arising in solution earlier and in a more sensitive way.

It may be premature to comment in detail on the significance of the data obtained. They do indicate specific interaction of ions and a neat division into two groups. In this sense the result appears to be clearer than the effect, for instance, of similar electrolytes on the thermal transition of DNA.$^{10}$ It contradicts a statement$^{11}$ that "Mn$^{2+}$ is the least effective of the three common divalent ions in condensing chromatin". With some care a variety of significant experiments under near physiological conditions can be executed.

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