

Critical cation balance in B \rightarrow Z transition: role of Li⁺

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The sodium salt of poly(dG-dC) is known to exhibit a B \rightarrow Z transition in the presence of various cations and 60% alcohol. We here show that the lithium salt of poly(dG-dC) does not undergo B \rightarrow Z transition in the presence of 60% alcohol since Li⁺ with its large hydration shell cannot stabilize the Z-form. On the other hand, high concentrations of Mg²⁺ or micromolar concentrations of the cobalt hexamine complex which are known to stabilize the Z-form can compete with Li⁺ for charge neutralization and hence bring about a B \rightarrow Z transition in the same polymer. From the model building studies the mode of action of the cobalt-hexamine complex in stabilizing the Z-form is postulated.

B \rightarrow Z transition Circular dichroism Poly(dG-dC) Li salt Cobalt-hexamine binding

1. INTRODUCTION

Although double helical DNA is known to exhibit polymorphism under the influence of different cations [1] it is not very clear how exactly one cation stabilizes one form of DNA over the other under a given humidity condition. In vivo DNA exhibits different biological functions for which the presence of various cations in millimolar quantities is obligatory. With the discovery of left-handed Z-DNA [2,3] in alternating purine-pyrimidine sequences a renewed interest has developed to understand the exact role of cations in stabilizing various secondary structures of DNA.

The B \rightarrow Z transition in poly(dG-dC) has been shown to be promoted by some cations, ligands, solvent changes, topological stress and chemical modification of the polymer [4-6]. The molecular mechanism by which this transition occurs with the inversion in a helical sense is yet to be elucidated.

Authors in [5] have examined the effects of a variety of divalent and polyvalent cations on poly(dG-dC) and poly(dG-m⁵dC). To the best of our knowledge there is no report on the Li salt of poly(dG-dC). Earlier, all observations were made

in the presence of 50 mM NaCl, monitoring the change in the absorption spectrum to follow the B \rightarrow Z transition [5]. This raises a question as to whether Na⁺ is essential for the B \rightarrow Z transition, brought about by alcohol, divalent and polyvalent cations. We here report a study on Li poly(dG-dC) and of the requirement of the critical ionic balance in controlling the B \rightarrow Z transition with change in solvent polarity.

2. MATERIALS AND METHODS

The sodium salt of poly(dG-dC) was obtained from PL Biochemicals. To prepare the Li salt of poly(dG-dC), advantage was taken of the fact that Li⁺ has a higher binding affinity than that of Na⁺; 500 μ g of poly(dG-dC) was dissolved in a solution containing 10 mM of LiCl, pH 6.5. The solution was then dialyzed against 3 mM LiCl extensively to replace all bound Na⁺ by Li⁺. The presence of Na⁺ was checked using a flame photometer attached to a sodium filter. When the sample was completely free of Na (<0.01 mM), it was dialyzed against quartz-distilled water to remove excess free Li⁺. At this stage the sample had a 1:1 ratio of Li:phosphate. This sample was then lyophilized and dissolved in an appropriate solution for further CD

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measurement. CD measurements were done using a JASCO J-20 spectrophotometer with a temperature attachment.

3. RESULTS

The CD spectrum of Li poly(dG-dC) in water is shown in fig.1. The spectrum is similar to that exhibited by the Na salt of poly(dG-dC) under low ionic strength. The positive band around 270 nm with a shoulder at 282 nm is much weaker compared to the negative band at 248 nm. This characteristic CD spectrum of poly(dG-dC) is slightly different from the conservative spectra observed for natural DNA from various sources. Addition of 40% alcohol enhanced the positive band at

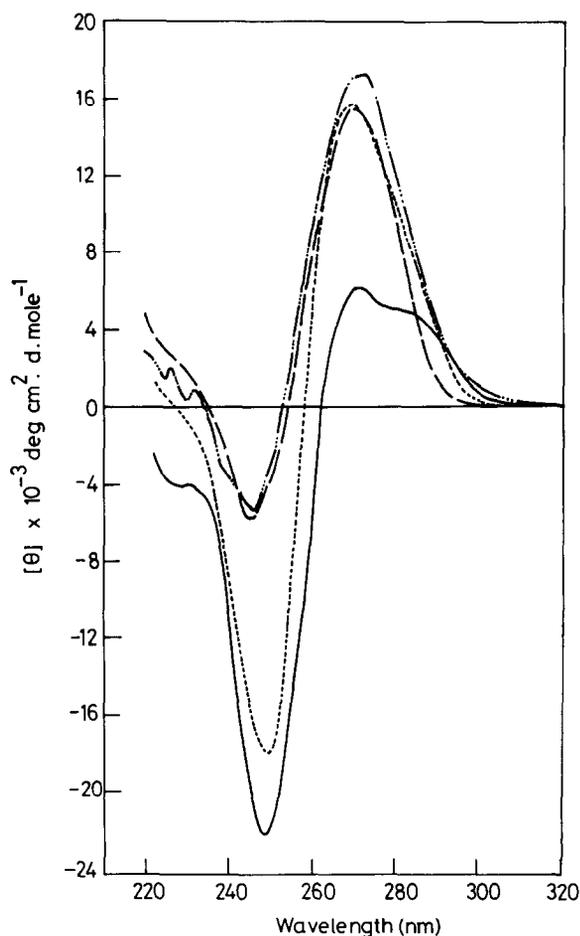


Fig.1. CD spectra of Li salt of poly(dG-dC) in: aqueous solution (—); 40% ethanol (-----); 60% ethanol (- · - · -); 80% ethanol (- · - · -).

270 nm with the loss of the 282 nm shoulder and the spectrum became almost conservative (fig.1). Alcohol titration of the Li salt of poly(dG-dC) did not show any inversion of the CD spectrum at 50–60% alcohol, unlike the sodium salt of the same polymer [7]. The Li polymer in 60% alcohol gave a strong positive band around 270 nm with a weak negative band around 245 nm (fig.1). Overall the spectra resemble the A-form CD spectra [7]. Even in the presence of 80% alcohol the spectra remained in the A-form (fig.1). To make sure that cation concentration did not limit the expected B → Z transition, 3 mM LiCl was added in the presence of 60% alcohol. There was only a reduction in the positive band; otherwise the CD spectrum remained unaltered indicating that the Li salt of poly(dG-dC) does not undergo a B → Z transition in the presence of alcohol. In order to see whether Li⁺ locks poly(dG-dC) in the B-form or, fails to stabilize the Z-form even when the Z-form is favoured through decrease in water activity, the following experiment was performed. The Z-form of poly(dG-dC) was stabilized by dissolving the Na salt of the polymer in 60% alcohol and equilibrating at low temperature (4°C) for several hours. To this solution LiCl of known concentration was added. Addition of 4 mM of Li destabilized the Z-form as evident from the drastic reduction in the 290 nm negative band intensity (fig.2). Other than alcohol, divalent cations like Mg²⁺ at high concentrations and polyvalent cations like Co(NH₃)₆³⁺ in micromolar concentrations are known to induce a B → Z transition in the Na salt of poly(dG-dC) [5]. Although alcohol failed to induce the B → Z transition in the Li salt of poly(dG-dC), both 700 mM Mg²⁺ and 0.036 mM Co(NH₃)₆³⁺ could induce a B → Z transition as observed by the CD spectra (fig.3). However, the Li salt of poly(dG-dC) in the presence of 700 mM Mg²⁺ does not exhibit a canonical Z-DNA CD spectrum. In the presence of 0.02 mM Co(NH₃)₆³⁺, the B → Z transition is not complete (fig.3). It is interesting to note that the Li salt of poly(dG-dC) required a higher concentration (0.036 mM) of Co(NH₃)₆³⁺ as compared to the Na salt of the polymer (0.02 mM) [5] to drive the structure completely to the Z-form.

These results clearly indicate that though Li⁺ has higher affinity to stabilize the B-form it does not lock the structure in the B-form to prevent a B → Z transition. It is more plausible that once there is

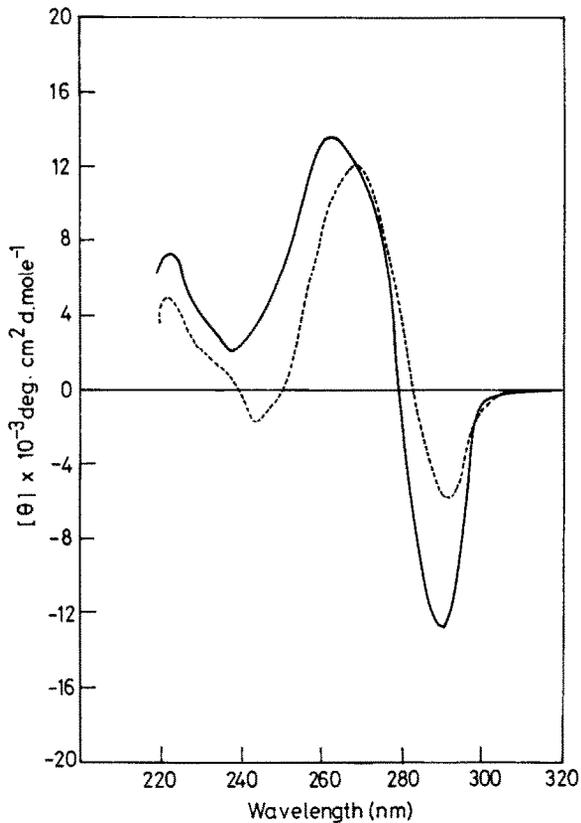


Fig.2. CD spectra of Na salt of poly(dG-dC) in: 60% ethanol (—); 60% ethanol; and 4 mM LiCl (-·-·-·-).

another cation which can stabilize the Z-form and can compete with Li^+ for charge neutralization, a B \rightarrow Z transition takes place. Hence, our results clearly show that Li^+ with its large hydration shell cannot stabilize the Z-form by binding to phosphates.

In order to understand the role of cobalt complexes in stabilizing the Z-form, we measured the concentration required for a complete B \rightarrow Z transition with changes in ligand composition. It is interesting to note that the concentration of salt required to induce the B \rightarrow Z transition increased from $\text{Co}(\text{NH}_3)_6\text{Cl}_3 < [\text{Co}(\text{NH}_3)_4\text{H}_2\text{OCl}]\text{Cl} < [\text{Co}(\text{en})_2\text{H}_2\text{OCl}]\text{SO}_4 < \text{CoCl}_2$. The concentration required is given in table 1.

Since free CoCl_2 requires a much higher concentration (3 mM) for the transition in the case of the Na salt of poly(dG-dC) [8] it is obvious that other than charge neutralization through cations, the

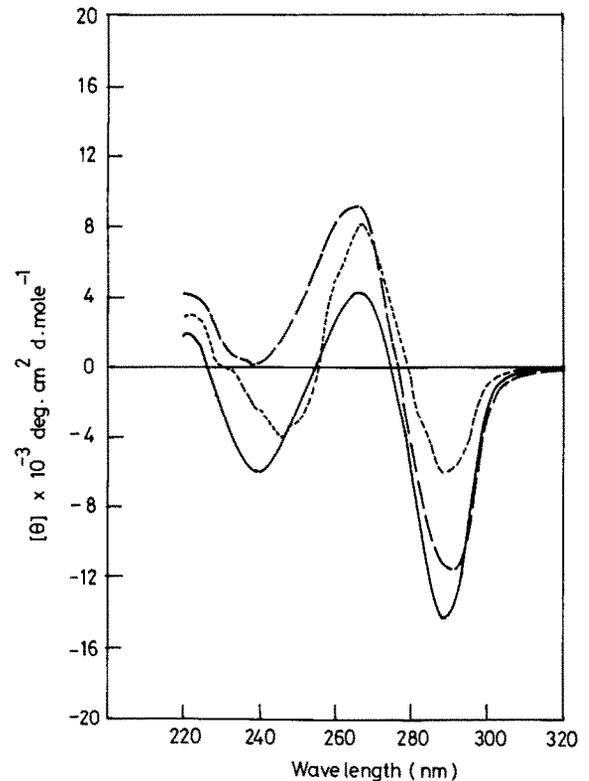


Fig.3. CD spectra of Li salt of poly(dG-dC) in: 700 mM MgCl_2 (—); 0.02 mM $[\text{Co}(\text{NH}_3)_6]\text{Cl}_3$ (-·-·-·-); 0.036 mM $[\text{Co}(\text{NH}_3)_6]\text{Cl}_3$ (-·-·-·-).

ligands of these complexes may interact with the exposed sites of the bases like N7 of G in the Z-helix. Recently $\text{Co}(\text{NH}_3)_6^{3+}$ has been extensively used for the B \rightarrow Z transition. So to examine the possible location of $\text{Co}(\text{NH}_3)_6^{3+}$ -binding, we constructed a molecular model of $\text{Co}(\text{NH}_3)_6\text{Cl}_3$ and

Table 1

Concentration of the cobalt complex required for a B \rightarrow Z transition of Li salt of poly(dG-dC)

Substance	Concentration (mM)
$\text{Co}(\text{NH}_3)_6\text{Cl}_3$	0.02
$[\text{Co}(\text{NH}_3)_4\text{H}_2\text{OCl}]\text{Cl}$	0.04
$[\text{Co}(\text{en})_2\text{H}_2\text{OCl}]\text{SO}_4$	0.1
$^a\text{CoCl}_2$	3.0

^a Na salt of poly(dG-dC)

poly(dG–dC) in the Z-form [9,10]. From model building studies it can be concluded that the amine function of the cobalt complex can bind through hydrogen bonding. This hydrogen bond interaction may be through the pendant oxygens of the two polynucleotide chains or through N7, O6 of G and phosphate of the next residue in the 5' direction. It is interesting to note that in the latter case, the orientation of the N7 and O6 of G and phosphate of the next residue in the 5' direction is such that it can favourably interact with the 3 amine functions of the cobalt complex, in two perpendicular planes (fig.4). Thus the *syn* conformation of G in the Z-helix facilitates the binding and thereby stabilizes it at a very low concentration of the hexamine–cobalt complex. The experimental data of both divalent and trivalent cobalt–amine complexes are in agreement with model-building studies.

4. DISCUSSION

It is believed that transition from B-DNA to another form, whether to the A- or the Z-families or to the C-helix within the same family, occurs by lowering the activity coefficient or the effective concentration of the surrounding water molecules [11]. The lowering of the actual concentration can be brought about by drying or replacing water by another solvent. This can also be achieved in the presence of cations which effectively decrease water activity by binding water molecules.

In low ionic strength, the formation of an ion-pair between the phosphate group of DNA and the counter-ion as predicted by the electrostatic theories is accompanied by a new distribution of electrical potential [12]. There is a consecutive rearrangement of water molecules around the ion-pair; some molecules of water will go from the close packaging inside the hydration shell to the normal open structure of water. Li^+ has a much greater covalent character than other alkali metals. In solution, the small ionic radius and high charge density in a large hydration sphere with a diameter the order of 14.8 Å [13]. It has been suggested that because of its high polarizing power, Li^+ might cause distortion in the electron cloud of ligands. In addition, the ion has a high affinity for oxygen and nitrogen sites as compared to that of the other metals of Group I.

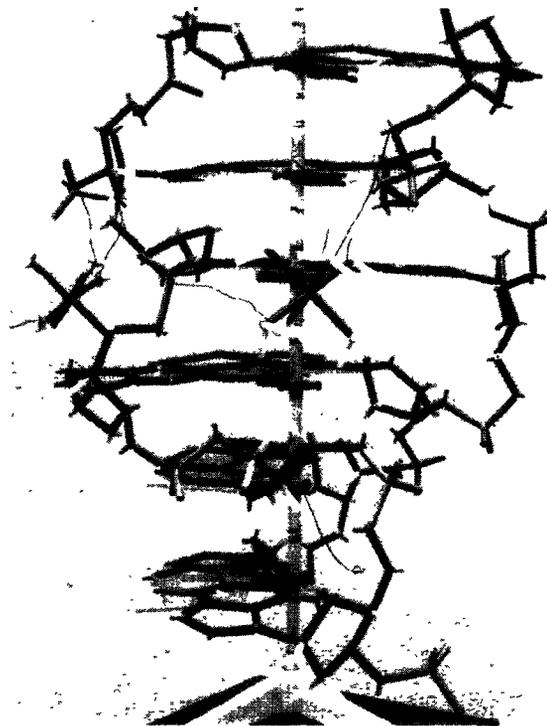


Fig.4. Binding of cobalt hexamine to Z-DNA.

In the presence of alcohol, the binding of Li^+ is enhanced by the fact that the ionic interaction of Li^+ increases in non-aqueous solutions as the hydration shell is removed. This may then prevent the transition of poly(dG–dC) in the presence of alcohol. Mg^{2+} , which has an atomic size similar to that of Li^+ but a higher ionization potential, completes and exchanges with Li^+ . With increase in concentration of Mg^{2+} there is a shift in equilibrium towards Z-form.

The initiation of an event by the conformational change of DNA, induced by metal-binding, ultimately must be reversed by release of the metal from its binding site. Such variation in binding of the cation may be induced by the local molecular environment. This can happen *in vivo* by local changes in the concentration of other ions, including proteins. Since weakly binding species are more susceptible to small changes in local conditions, fine regulation might be achieved by small, and therefore metabolically controlled, changes in metal concentration.

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