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Inversion of DNA helicity induced by zinc(II)–macrocyclic polyamine complexes

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Exposure of synthetic polynucleotide poly(dG-dC)·poly(dG-dC) to Zn^{II} cyclen, 2 (cyclen = 1,4,7,10-tetraazacyclododecane), produces a dramatic change in its circular dichroism (CD) spectrum in H_2O at pH 7.2, 24°C: the CD spectrum of the initial B form changes to that of the Z form (or a non-Z structure with a left-handed helix) at very low concentrations ([Zn^{II}]/[base pair] in molar basis ≤ 1). By contrast, Zn^{II}–[12]aneN$_3$, 1 ([12]aneN$_3$ = 1,5,9-triazacyclododecane), and Zn^{II}–cyclam, 3 (cyclam = 1,4,8,11-tetraazacyclotetradecane), do not significantly have such a topological affect on the polynucleotide even at much higher concentrations. An increase in Na^+ ionic strength nullified the effect of 2 on the CD spectrum, indicating an outside interaction (electrostatic and/or hydrogen bonding) of the DNA model. This study illustrates the significance of the macrocyclic ligand structure around the Zn^{II} ion for specific interaction with DNA.

INTRODUCTION

The interaction of metal ions or complexes with DNA has been attracting much interest with relevance to DNA–drug interactions, DNA modifications, and DNA regulatory processes. In recent years much effort has been devoted to the design and synthesis of metal complexes that recognize, bind to, and cleave DNA.[1] Naturally occurring and artificially designed agents have often shown site specificity in their reactions with DNA through intercalative, groove-binding, or hydrogen bonding interactions.[2–4] Previously, we demonstrated that protonated macrocyclic polyamines selectively interact with mononucleotides (e.g. ATP) through electrostatic and hydrogen bonding interactions.[5] These results led us to consider that macrocyclic polyamine metal complexes could be a new type of DNA binding agent. Until now, the investigation of macrocyclic polyamine complexes that effect specific DNA binding remains scarce.[6]

Recently we have used macrocyclic polyamine ligands to study intrinsic acidic properties of the Zn^{II} ion to elucidate the role of Zn^{II} in zinc enzymes.[7–11] These Zn^{II} complexes can mimic various biologically important reactions such as hydrolysis,[8,9] hydration of carbonyls,[8] and alcohol dehydrogenase-like 'hydride transfer' reactions.[11] Most interestingly, the catalytic activities are quite sensitive to variations in the acidity of Zn^{II}, which is elicited by the co-ordination environment around Zn^{II}. For instance, the Zn^{II} ion in [12]aneN$_3$ possesses an outstanding affinity to biologically important anions (HCO_3^-, CH_3COO^-, HPO_4^{2-} and Cl^-),[10] which led us to examine the binding of the Zn^{II} macrocyclic polyamine complexes to biopolymers bearing anionic phosphate groups.

In this paper, we examine circular dichroism (CD) spectral changes upon binding of a series of Zn^{II} macrocyclic polyamine complexes, Zn^{II}–[12]aneN$_3$, **1** ([12]aneN$_3$ = 1,5,9-triazacyclododecane), Zn^{II}–cyclen, **2** (cyclen = 1,4,7,10-tetraazacyclododecane), and Zn^{II}–cyclam, **3** (cyclam = 1,4,8,11-tetraazacyclotetradecane), with poly(dG-dC)·poly(dG-dC) and poly(dA-dT)·poly(dA-dT). We discovered for the first time that among the three congeners Zn^{II}–cyclen complex, **2**, alone induces a dramatic transition from right-handed B form to putative left-handed Z form of poly(dG-dC)·poly(dG-dC) at very low concentrations.

EXPERIMENTAL SECTION

Poly(dG-dC)·poly(dG-dC) and poly(dA-dT)·poly(dA-dT) were purchased from Pharmacia. Extinction coefficients used to determine the polynucleotide concentrations were as follows: poly(dG-dC)·poly(dG-dC), $\varepsilon_{254} = 8.4 \times 10^3$ M(P)$^{-1}$ cm^{-1};[12] poly(dA-dT)·poly(dA-dT), $\varepsilon_{262} = 6.6 \times 10^3$ M(P)$^{-1}$ cm^{-1}.[13]

([12]aneN$_3$–Zn^{II}–OH)$_3$·(ClO$_4$)$_3$·HClO$_4$, **1**,[18] ([12]aneN$_4$–Zn^{II}–OH$_2$)·(ClO$_4$)$_2$, **2**,[14] and ([14]-aneN$_4$–Zn^{II})·(ClO$_4$)$_2$, **3**,[15] were prepared as previously described.

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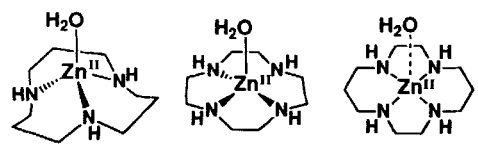
UV spectral experiments were conducted with a Hitachi U-3200 spectrophotometer at 25°C. CD spectra were recorded on a JASCO 720 spectropolarimeter. Polynucleotides, typically *ca.* 0.1 mM, were contained in a 10 mm path length quartz cell. The CD spectra were recorded in the 350–210 nm range at 24°C. Then aliquots of a solution of each Zn^{II} complex in H_2O at pH 7.2 were added and the spectrum measured after each addition until $r \sim 10$ ($= [Zn^{II}]/[\text{base pair}]$) was achieved at the end of titration. CD is expressed in terms of molar ellipticity $[\Theta]$ (deg·cm^2/dmol).

RESULTS AND DISCUSSION

Pohl and Jovin[16a] first described two conformations of DNA which were found in a sequence containing alternating guanine and cytosine residues. They revealed that poly(dG-dC)·poly(dG-dC) undergoes the transition from the B form (right-handed) to the Z form (left-handed) at high ionic strength (above 2.5 M NaCl or 0.7 M $MgCl_2$) accompanied by altered CD and absorption spectra.[16] This finding was supported by the X-ray structural analysis of a synthetic double helical fragment of DNA containing six base pairs with the sequence d(CpGpCpGpCpG) as a left-handed Z form with Watson–Crick base pairs.[17] This differs significantly from the familiar right-handed helical B form.

A transition of double helical DNA depends strongly on the nature and the concentration of coexisting cationic species as well as the solvent and the temperature. A number of cations (Na^+, K^+, Li^+, Mg^{2+}, Ca^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+} and Zn^{2+}, etc.) have been known so far to induce B ↔ Z transitions in DNA by a co-operative intramolecular process.[18] However, they need to be present at very high and non-physiological concentrations (except for Hg^{2+} [19] and $Co(NH_3)_6^{3+}$,[16b] etc.).



Zn^{II}-[12]aneN$_3$ **1** Zn^{II}-cyclen **2** Zn^{II}-cyclam **3**

To elucidate the structural prerequisites of agents effecting specific interaction with DNA, systematic structural modification of metal complexes may serve as a useful tool. We thus initiated an investigation of changes in the absorption and CD spectra to monitor the transition of poly(dG-dC)·poly(dG-dC) upon the addition of three Zn^{II} macrocyclic polyamine complexes, **1**–**3**. These Zn^{II} complexes are thermodynamically and kinetically stable at physiological pH (the complexation completes at lower pH), and thus the structures are rigid and well-defined in aqueous solution.[8]

CD titrations of alternating synthetic polynucleotide, poly(dG-dC)·poly(dG-dC) [9.7×10^{-5} M(P)], with Zn^{II}–cyclen, **2**, resulted in little change in its UV absorption; however a dramatic change in its CD spectrum in H_2O at pH 7.2, 24°C was observed. The UV absorption spectra at $r = 0$ and 1 ($r = [Zn^{II}]/[\text{base pair}]$ for duplexes in molar basis) and the CD spectral changes at various concentrations of **2** ($0 \leqslant r \leqslant 1$) are illustrated in Figures 1 and 2, respectively. The CD spectral changes are quite similar to those at high concentrations of NaCl or $MgCl_2$. However, most interesting is the transition that

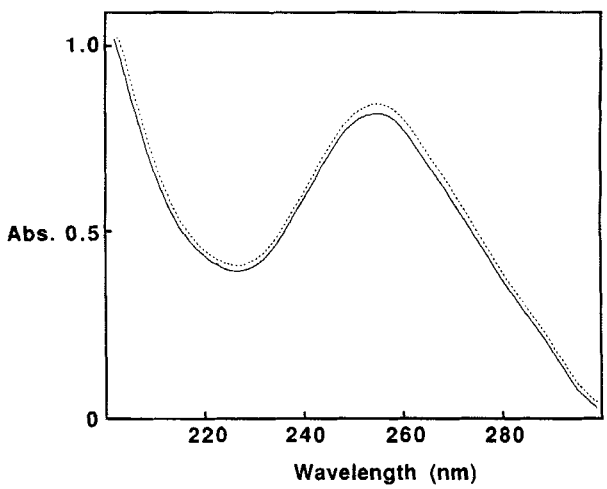


Figure 1 UV absorption spectra of poly(dG-dC)·poly(dG-dC) in H_2O (9.7×10^{-5} M) at pH 7.2, 25°C, in the absence ($r = 0$, solid line) or in the presence of **2** ($r = 1$, broken line). $r = [2]_{added}/[\text{GC base pair}]$.

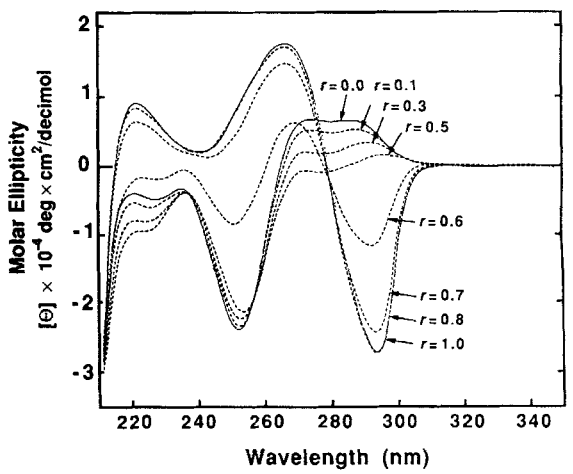


Figure 2 CD spectra of poly(dG-dC)·poly(dG-dC) in H_2O (9.7×10^{-5} M) at pH 7.2, 24°C, in the absence or in the presence of **2**. $r = [2]_{added}/[\text{GC base pair}]$.

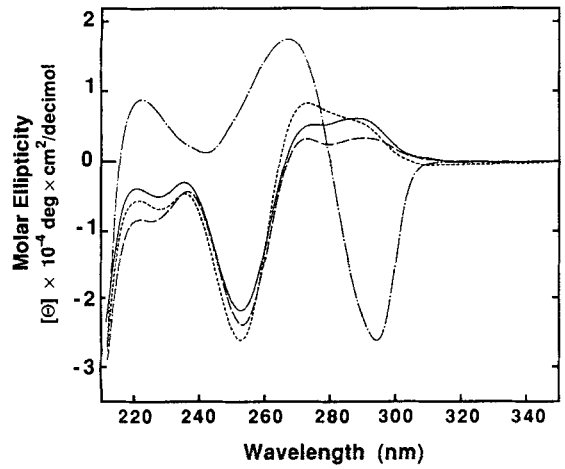


Figure 3 CD spectra of poly(dG-dC)·poly(dG-dC) at pH 7.2, 24°C in H_2O ($r = 0$, solid line; $r = 1$, dotted broken line), in 10 mM $NaClO_4$ ($r = 1$, broken line), and in 50 mM $NaClO_4$ ($r = 1$, dotted line). $r = [2]_{added}/[GC\ base\ pair]$.

completes at $r = 1$, where the negative charges on backbone phosphates are effectively neutralized by the positively charged Zn^{II} [where the H_2O bound to Zn^{II} ($pK_a = 8.0$)[8] is not deprotnated]. These phosphate groups come very close together in the Z form. Further addition of **2** (to $r \sim 10$) does not affect the change any more. In this way, Zn^{II}–cyclen, **2**, has a potent effect on the thermodynamic stability of the putative Z form (or a non-Z structure with left-handed helix) of poly(dG-dC)·poly(dG-dC). However, identification of a left-handed helical structure must be made with caution until the transition can be demonstrated in similar solvents by means of X-ray diffraction of the polymer fibre.

We then examined the structurally related Zn^{II} macrocyclic polyamine complexes, Zn^{II}–[12]aneN$_3$, **1**, and Zn^{II}–cyclam, **3**. Unlike **2**, these hardly affected the poly(dG-dC)·poly(dG-dC), even at much higher concentrations ($0 \leqslant r \leqslant 10$).

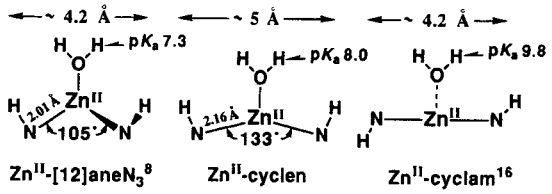
An increase of Na^+ ionic strength to 50 mM nullified the effect of **2** on the stabilization of the Z form as shown in Figure 3, indicating a surface interaction (electrostatic and/or hydrogen bonding) between **2** and the DNA model. From our recent work,* it was predicted that **2** had no chance of interacting with nucleic bases dG and dC in an aqueous solution.

To account for our results, we looked at the findings obtained from the X-ray analysis of the hexamer d(CpGpCpGpCpG).[17] They revealed that distribution of backbone negative charges is strikingly different in the right-handed B form and in the left-handed helix. A small cation, even when it is hydrated, may form stabilizing bridges between the close pairs of phosphodiester groups on the Z form backbone or between a base and a phosphodiester. In crystals of the hexamer with Mg^{2+}, Mg^{2+} forms a stabilizing bridge between N7 of guanine and a phosphodiester oxygen through a water molecule.[17] There the backbone assumes a so-called Z_{II} conformation which differs from the common Z_I conformation by rotation of phosphate groups corresponding to altered ribose-phosphate torsion angles. With a relatively bulky Zn^{II}–cyclen complex such an interaction is hardly conceivable. Hence, a phosphodiester group with a regular Z_I conformation, where P–O bonds are directed outside, may readily interact with the Zn^{II}–cyclen complex, **2**.

More interestingly for the deoxyguanosine residues in Z-DNA, the ribose takes a C3′-*endo* conformation, while the ribose in B-DNA adopts the C2′-*endo* conformation.[20,†] This difference significantly affects the intermolecular phosphorus–phosphorus distances. That is, the distance of ~7.0 Å in C2′-*endo* ribose is reduced to ~5.9 Å in C3′-*endo* ribose.[21] As shown below, Zn^{II}–cyclen, **2**, from X-ray crystal data,[‡] possesses four NH moieties as hydrogen bonding donors (where the electron densities of H atoms are favourably reduced due to N-co-ordination to Zn^{II}) with a diagonal distance of ~5 Å, and the H_2O ($pK_a = 8.0$)[8] bound to the central Zn^{II} may participate in the hydrogen bonding.

The conformation of the Zn^{II}–cyclen complex, **2**, results in an orientation in which all of the NHs lie along the same side of the macrocycle. Such a situation creates a hydrophilic face and consequently a corresponding hydrophobic region on the opposite face. The relative interaction(s) of these two faces with the DNA backbone may be an important structural factor for macrocyclic polyamine complexes in general. Zn^{II}–[12]aneN$_3$, **1**, and Zn^{II}–cyclam, **3**, thus do not exhibit similar facial differentiation.

Finally, we propose a possible interaction between Zn^{II}–cyclen, **2**, and the backbone phosphate in Z form



*In an aqueous solution, Zn^{II}–cyclen, **2**, hardly interacts with dG, dC and dA, and selectively binds to dT. Detailed results will be reported elsewhere.

†Two major conformations are found for the GpC phosphate groups in Z-DNA.

‡Recently, we isolated azidothymidine–Zn^{II}–cyclen complex and performed its X-ray crystal analysis. Two hydrogen bonding are observed between two amine nitrogens of the ligand and two carbonyl oxygens of the thymidine base. The hydrogen bonding also play a crucial role in stabilization of the complex in aqueous solution. The details will be reported elsewhere.

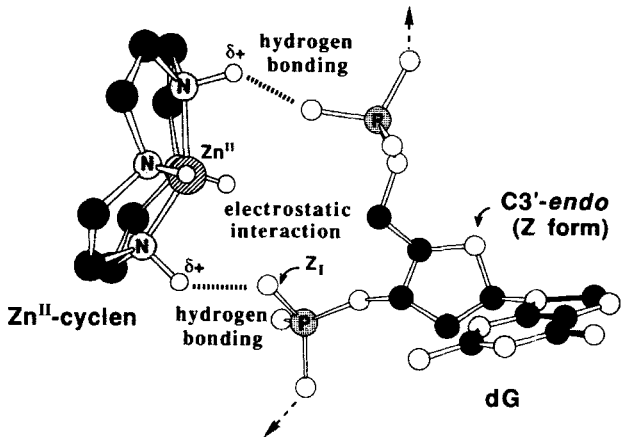


Figure 4 A possible interaction between Zn^{II}–cyclen, **2**, and the backbone phosphate groups of poly(dG-dC)·poly(dG-dC) in Z form. The conformation of the deoxyguanine unit including phosphate groups is based on the X-ray structure of hexamer d(CpGpCpGpCpG) given in ref 17(a).

of poly(dG-dC)·poly(dG-dC), based on the above discussion and the X-ray structure of the hexamer d(CpGpCpGpCpG) as shown in Figure 4. There is a suitable distance between two amine protons (~5 Å) and the arrangement of the functional groups in **2** results in the two forming favourable hydrogen bonds with the close pairs of phosphodiester oxygens of C3'-*endo* ribose of deoxyguanine in Z form, as well as the electrostatic interaction.

We are currently examining the interaction of macrocyclic polyamine complexes with poly(dA-dT)·poly(dA-dT). Preliminary results indicate a different binding mode. Whether this difference is based on DNA conformation or on differences in specific base pair interaction awaits further experiment.

This study illustrates the significance of the macrocyclic ligand structure around the Zn^{II} ion for specific interaction with DNA.

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