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Zinc Z-DNA

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ABSTRACT

Circular dichroism spectra of poly(dG-dC) in the presence of some zinc complexes exhibit the characteristic inversion associated with the formation of a left handed helix. The transition of B to Z DNA is cooperative and slow. The concentration of zinc complex at the mid point of the transition is strongly dependent upon the nature of the ligand bound to zinc. The most efficient species is one with a tetradentate amine for which the mid point is observed at a zinc:nucleotide ratio of 1:24. ^{31}P spectra of one of these complexes confirm the presence of a left handed helix.

INTRODUCTION

The conformation, dynamics and genome expression of DNA appears to be strongly dependent upon its sequence. Probably the most striking sequence dependent property yet observed is the ability of certain alternating purine-pyrimidine sequences to change from a right handed B form to a left handed Z form. In high salt concentration (1) or ethanolic solution (2) poly(dG-dC) exists in a left handed or Z form which has since been observed in the crystal state (3,4). Methylation (5) or a combination of certain divalent metal ions in ethylene glycol solution (6-8) also promote the formation of Z DNA. It has been shown that right and left handed forms can exist in close proximity (9). Poly(dAm^{5}dC).poly(dG-dT) also exists in a left handed form under high salt conditions (10). In searching for physiologically attainable conditions under which the B→Z transition can take place we have examined the interaction of some zinc complexes with poly(dG-dC). Some of these complexes are shown to be extremely effecient in promoting the transition to Z DNA and do not require dehydrating conditions.

MATERIALS AND METHODS

Poly(dG-dC) was purchased from Boehringer, dialyzed against Chelex resin to remove divalent metal ions and then twice against 2mM sodium cacodylate, pH 7.1. The zinc complexes were prepared by mixing equimolar quantities of zinc chloride and the appropriate ligand, see Table 1, in 2mM buffer. These solutuions (10^{-2} or 10^{-3}M) were adjusted to pH 7.1. Titrations were carried out at 35°C by addition of microliter quantities of these stock solutuions. At this temperature equilibrium was attained after 30-40 minutes. Circular dichroism spectra were recorded on a Jobin Yvon Mark V dichrograph.

Kinetic measurements were made by observing the change in the UV spectrum at 295nm.

^{31}P NMR spectra were recorded at 202 MHz on a Bruker WM500. The polynucleotide (1 O.D., 140 μM in nucleotide) was dissolved in D_2O, 2mM in buffer pH 7.1 and titrated with the Zinc-cysteine complex until the CD spectrum showed 80% Z form. The spectrum of this solution corresponds to 120,000 scans and was obtained at 30°C. Chemical shifts are referenced to external trimethyl phosphate.

RESULTS AND DISCUSSION

The changes induced in the circular dichroism (CD) spectra of poly(dG-dC) upon titration with a 1:1 complex of zinc chloride -diethylenetriamine (Zn dien) are shown in Fig. 1. In the first stage the two positive bands almost disappear after which at higher Zn-dien concentrations a cooperative transition with an isobestic point at 278 nm is seen leading to the inverted spectrum typical of the Z form. The mid point of the transition occurs at ca. 40 μM Zn or approximately one zinc complex molecule per base pair. Substituting a tetradentate amine, tris (2-aminoethyl) amine for diethylenetriamine has a very pronounced effect upon the formation of the Z form. The mid point of the transition occurs at 3 μM zinc complex or a zinc to base pair ratio of 1:12. On doubling the DNA concentration (140 μM nucleotide) the mid point is found at a ratio of 1:18. Thus at the mid point the length of cooperativity is at least one turn of the double helix (11). These zinc complexes are more power-

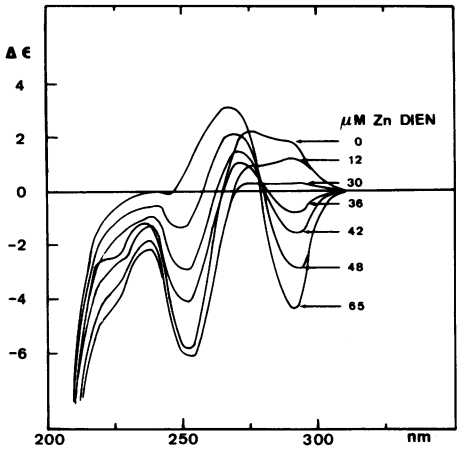

Fig. 1. Circular dichroism spectra for the titration of poly(dG-dC) 7 x 10^{-5}M, pH 7.1, 2 mM sodium cacodylate with a 1:1 complex of zinc chloride and diethylenetriamine.

ful in inducing the Z form than Mn(II) or other divalent metal ions (6-8).

Zinc chloride does modify the CD spectrum of poly(dG-dC) as shown in Fig.2. However the spectrum of the limiting form shows only a small negative band which is shifted to higher wavelengths. The transition B→Z is also much less cooperative than for all the other zinc complexes. We cannot yet be sure if this corresponds to perhaps the other extreme of a family of Z forms or to yet a different conformation. The effect of different ligands bound to the zinc changes both the final spectrum, Fig. 2, and the concentration of zinc complex required to obtain 50% Z form, Table I. Comparing the amine ligands, there is a

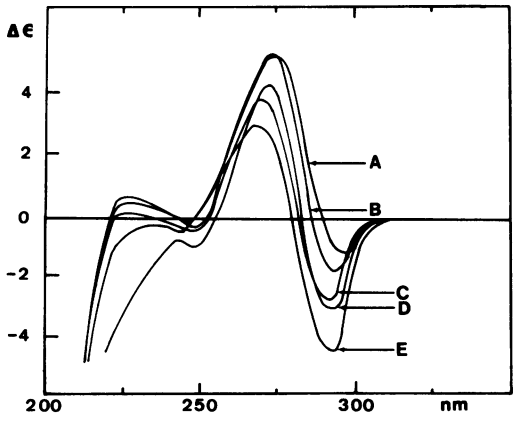

Fig. 2. Circular dichroism spectra of the limiting forms obtained upon titration with different zinc species : A : zinc chloride, and complexes (1:1) of zinc chloride with B. Glycine, C. Ethylenediamine D. Cysteine and E. Diethylenetriamine or tris (2-aminoethyl) amine.

TABLE 1
CONCENTRATIONS OF ZINC-LIGAND COMPLEXES
AT THE MID POINT OF THE B→Z TRANSITION

Ligand	Zn-ligand μM
Tris (2-aminoethyl) amine	3
Diethylenetriamine	42
Ethylenediamine	90
Glycine	118
Cysteine	80
Cysteine (poly(dG-dC) in 10mM NaCl)	120
Iminodiacetate	a
Nitrilotriacetate	b
Tris (2-aminoethyl) amine/20% ethylene glycol	1.0
Diethylenetriamine/20% ethylene glycol	2.5
--- (zinc chloride)	150

Poly(dG-dC), 7 x 10^{-5}M (nucleotide), 2mM sodium cacodylate, pH 7.1. Zn-ligand corresponds to a 1:1 mixture of $ZnCl_2$ and ligand a) Only the first step is observed, no further change up to 2mM. b) No change in optical properties observed up to 2 mM.

very pronounced effect upon passing from a bidentate to tridentate to tetradentate ligand, the latter being 30 times more efficient than ethylenediamine. As the overall charge of the zinc complex remains constant either water molecules bound to zinc interact in such a way as to favour a B form or the bound amine groups interact with the DNA to favour a Z form.

CD spectra alone are not sufficient to unambiguously assign a DNA as being in a left handed helix. ^{31}P NMR has been used (12-13) to characterize the Z form, the spectra reflecting the dinucleotide repeat unit. The ^{31}P spectrum of poly(dG-dC) and of the complex formed with Zn-cysteine (80% Z, 20% B) are shown in Fig. 3. The spectrum in the presence of zinc clearly shows a resonance shifted ca. 1.5 ppm downfield characteristic of the Z form. The shift is not due to coordinated zinc alone as a spectrum obtained at half the zinc concentration, which is just before the Z form begins to appear, showed no shift in the ^{31}P spectrum. All the resonances are broad because at 202 MHz relaxation is dominated by the chemical shift anisotropy mechanism (13). The rather poor signal to noise is explained by the very low concentration (140 μM in nucleotide) which was necessary. At higher concentrations aggregation occured as has been observed

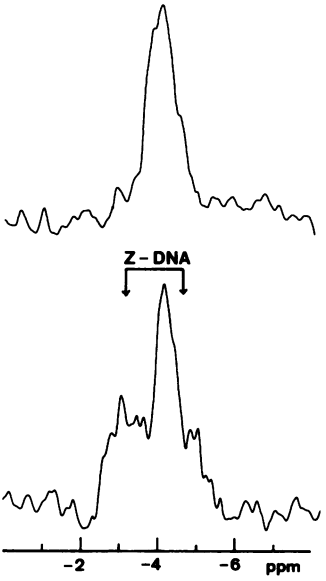

Fig. 3. ^{31}P NMR spectra of poly(dG-dC) at 140 μM (nucleotide), pH 7.1, 2 mM sodium cacodylate (upper) and of the same sample in the presence of 150 μM zinc-cysteine. Spectra were recorded at 30°C.

before in the presence of divalent metal ions (6). The titration was stopped at 80% Z form as this solution showed no aggregation over a 48 hour period.

As observed with the salt induced transition (1) we find that the reaction takes several minutes. Under pseudo first order conditions (10 fold greater concentration of zinc than that required to give 100% Z form), $t_{1/2}$ is 165s at 29.5°C for reaction with the zinc glycine complex. Analysis of the kinetic data as a function of temperature in the form of a Arrhenius plot gives an activation energy of 190 kJ mol^{-1}. This value is double that observed for the salt induced transition (1).

As has been observed with other divalent ions the presence of a dehydrating organic solvent has a synergistic effect upon the B→Z transition. In 20% ethylene glycol the mid point of the transition is observed at 2.5 μM zinc-dien or approximately one zinc complex molecule per two turns of the double helix. In the same solvent mixture the mid point for the zinc-tris (2-aminoethyl) amine complex is found at 1 μM Zn, Table 1. On the other hand increasing the salt concentration has an anti-cooperative effect upon the efficiency of the transition as shown for the zinc-cysteine complex in Table 1.

It has been observed (5) that the inert complex ion hexaminecobalt, $Co(NH_3)_6^{3+}$ also induces the Z form. However these zinc complexes interact by direct zinc coordination to the DNA. The bis complex $Zn(dien)_2^{2+}$ which has no free site for binding to the DNA has no effect upon the optical properties of the DNA up to a concentration of 1 mM Zn.

The role of the ligand bound to the zinc is not entirely clear. Neutral ligands seem to be more effecient and to give greater negative dichroism at 295 nm. However the overall charge on the zinc complex cannot be the only factor as the iminodiacetic acid complex was inefficient whereas the cysteine complex was the third most potent one found. This leads to questions concerning Z DNA-zinc bound ligand interactions in addition to direct zinc coordination and thus a possible role of zinc proteins in DNA conformational changes.

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