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B→Z DNA conformational changes induced by a family of dinuclear bis(platinum) complexes

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ABSTRACT

The reactions of bis(platinum) complexes of general formula $[\{PtCl_m(NH_3)_{3-m}\}_2(NH_2(CH_2)_nNH_2)]^{2(2-m)+}$ were studied with poly(dG-dC)·poly(dG-dC), poly(dG-m^{5}dC)·poly(dG-m^{5}dC) and poly(dG)·poly(dC). When m = 0 (Complexes II, n = 2,4) the complexes are saturated 4+ cations capable only of electrostatic interactions with the polynucleotide. Where m = 1 the complexes contain two monodentate platinum coordination spheres with the chloride trans to the diamine bridge (Complexes I, n = 2,4, 1,1/t,t). Complexes I give CD spectra characteristic of a 'Z-like' conformation upon reaction with poly(dG- dC)·poly(dG-dC) and poly(dG-m^{5}dC)·poly(dG-m^{5}dC) but not poly(dG)·poly(dC). The B→Z transition appears independent of interplatinum diamine chain length. As little as 1 bis(platinum) complex per 25 – 30 base pairs is sufficient to observe the Z-like spectrum. Covalent binding is however not a prerequisite for Z-DNA formation because the polyvalent cations II are also very effective in inducing the B→Z transition in either poly(dG-dC)·poly(dG-dC) or poly (dG-m^{5}dC)·poly(dG-m^{5}dC). In these cases, the concentrations of II required are significantly lower than analogous monomeric agents such as $[Co(NH_3)_6]^{3+}$. The possible biological consequences of the Z-DNA induction by bis(platinum) complexes are discussed.

INTRODUCTION

The simple inorganic compound cis-$[PtCl_2(NH_3)_2]$ (cis-diamminedichloroplatinum(II), cis-DDP, cisplatin) is currently the preferred agent used in the post-operative chemotherapeutic treatment of certain cancers (1). This drug has been found to be especially useful in the treatment of testicular, ovarian and carcinomas of the head and neck (2, 3). Cis-DDP mediates its cytotoxicity by forming DNA-DNA intrastrand and interstrand cross-links as well as protein-DNA cross-links in the cellular genome (4, 5, 6). The principal lesions caused by cis-DDP, guanine-guanine and adenine-guanine intrastrand cross-links, introduce a bend in the DNA helix which may block the progression of DNA polymerase during replication (7, 8). The less frequently occurring interstrand cross-link between two neighboring guanines has also been shown to be a block to RNA polymerase (9).

As effective as cisplatin has proven to be in cancer treatment, the drug displays a relatively limited spectrum of activity with associated toxicity, including peripheral nervous system damage, deafness and renal toxicity (1, 2, 3). Cisplatin chemotherapy has also been restricted by the selection of cisplatin-resistant tumor cells, especially with cancer cells that have been previously exposed to the drug. In light of these therapeutic limitations associated with cisplatin and the problem of acquired drug resistance, there exists a demand for a further generation of complexes which can circumvent these limitations.

To this end, our laboratory has synthesized a new class of dinuclear platinum complexes that are non-cross-resistant in both murine and human tumor cells (10, 11, 12). The original bis(platinum) complexes contained two cis-DDP units linked by a diamine chain of variable length, $[\{cis\text{-}PtCl_2(NH_3)\}_2H_2N(CH_2)_nNH_2]$ (2,2/c,c) (13). The development of this series was motivated both by the consideration that a greater conformational change on DNA might be reflected in increased antitumor activity and by the possibility that an altered sequence specificity for the bis(platinum) complexes relative to cis-DDP could be generated. The complexes $[\{cis\text{-}PtCl_2(NH_3)\}_2H_2N(CH_2)_nNH_2]$ are tetrafunctional and produce a complex array of DNA adducts including interstrand cross-links by binding of one Pt atom to each strand of DNA (14). To examine the properties of this unique bimetallic interstrand cross-link we next prepared a bis(platinum) complex containing monodentate coordination spheres, $[trans\text{-}\{PtCl(NH_3)_2\}_2H_2N(CH_2)_nNH_2]Cl_2$ (1,1/t,t) (12). In the absence of a bidentate coordination sphere, this compound is incapable of forming any cis-DDP-like lesions and allows delineation of the structural features of these bis(platinum) complexes that are critical for their biological activity (15, 16). In fact, $[trans\text{-}\{PtCl(NH_3)_2\}_2H_2N(CH_2)_4NH_2]Cl_2$ was found to be relatively more cytotoxic in both murine tumor L1210 and human ovarian carcinoma cells rendered resistant to cisplatin than in the sensitive parent cell lines (12).

In addition to the altered cytotoxicity exhibited by these bis(platinum) complexes, they display a range of DNA adducts with different sequence preferences in comparison to cis-DDP-

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DNA adducts (12). Unlike cis-DDP, alternating purine (G)-pyrimidine (C) runs were found to be reactive with the bis(platinum) compounds (12). Circular dichroism measurements on CT DNA modified by [trans-{$PtCl(NH_3)_2$}$_2$$H_2N(CH_2)_4NH_2$-]$Cl_2$ showed an unusual conformational change specific to this complex. To examine the nature of this change in more detail we have studied the effects of bis(platinum) complex binding to poly(dG-dC)·poly(dG-dC) and poly(dG)·poly(dC). We report in this paper on the unique property of certain bis(platinum) complexes to induce a B→Z transition in the topology of DNA with repeating CpG sequences.

MATERIALS AND METHODS

Synthesis of complexes

The complexes [trans-{$PtCl(NH_3)_2$}$_2$$H_2N(CH_2)_nNH_2$]$Cl_2$ (1,1/t,t, n = 2,4) were prepared by literature procedures (15). The tetra-amine complexes [{$Pt(NH_3)_3$}$_2$$H_2N(CH_2)_nNH_2$]$Cl_4$ (0,0, n = 2,4) were prepared by treatment of the 1,1/t,t complexes with NH_3 (aq) (17) and worked up as previously described for the synthesis of the n = 4 derivative (18). The n = 4 derivative had spectroscopic properties consistent with those previously reported and the hitherto unreported n = 2 derivative had analytical and spectroscopic properties fully consistent with its formulation.

Treatment of DNA with bis(platinum) complexes

A stock solution of the complexes (1 mM) was prepared fresh just prior to use by dissolving a complex in a Tris (10 mM), EDTA (1 mM) and NaCl(10 mM) buffer (1×TEN). This stock solution was further diluted using 1×TEN buffer to various concentrations to a final volume of 4 ml. The solutions of poly(dG-dC)·poly(dG-dC), poly(dG-m^{5}dC)·poly(dG-m^{5}dC) and poly(dG)·poly(dC) were prepared at a concentration of 16 μg/ml. The DNA solutions were added to each bis(Pt) complex dilution and incubated in the dark at 37°C for an hour. The reactions were quenched by raising the salt concentration to 100 mM using 5 M NaCl and placing the reactions on ice. Each sample was then extensively dialyzed overnight against cold (4°C) 1×TEN buffer to remove any unbound Pt complex. The final concentration of DNA in each sample was verified by UV/Vis spectral analysis using a Perkin-Elmer Lambda 6 UV/VIS Spectrophotometer.

Circular Dichroism spectroscopy

The Circular Dichroism studies were performed in a 3-cm circular quartz cell using a Jobin-Yvon Autodichrograph Mark V spectrophotometer. Each bis(platinum)-DNA sample was allowed to warm up to room temperature prior to placing a 3 ml sample into the cell for analysis. Each sample was scanned in the range 210−360 nm. A CD spectrum was generated which represented the mean of 3 scans from which the buffer background had been electronically subtracted.

For the poly(dG-dC)·poly(dG-dC) experiments with the bis(platinum) tetra-amine **(0,0)** complexes **IIa** and **IIb**, a stock solution of the tetra-amine complex was made in 1×TEN buffer as above. The required dilutions of this stock solution in the same buffer were added to the appropriate DNA solution (16 μg/mL) also in 1×TEN buffer. For poly(dG-m^{5}dC)·poly(dG-m^{5}dC) the buffer used was 0.15×TEN containing Tris (1.5 mM), EDTA (0.15 mM) and NaCl (1.5 mM). The concentration was determined from UV Vis spectroscopy as 80 μM and the appropriate concentration of [{$Pt(NH_3)_3$}$_2$$H_2N(CH_2)_4NH_2$]$Cl_4$ (**IIb**) (40 μM) was dissolved in 0.1×TEN to give a Pt complex:Phosphate ratio of 1:2. For CD measurements, the samples were heated at 50−60°C for 10 minutes and then cooled to room temperature (22). The CD spectra were run immediately as above.

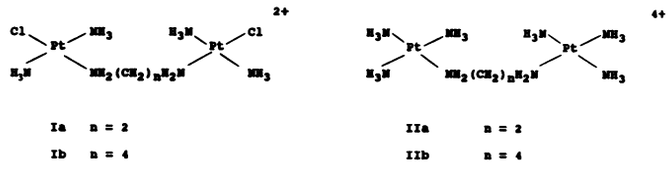


Figure 1. Structures of bis(platinum) complexes used in this study. For a convenient abbreviation of bis(platinum) complexes we have adopted a system where the numbers refer to the number of chlorides (or anionic leaving groups) on each platinum atom. For those possibilities where there is only one chloride in a coordination sphere, the lettering refers to the geometry with respect to the nitrogen of the bridging diamine. Thus **I**, [{trans-$PtCl(NH_3)_2$}$_2$$H_2N(CH_2)_nNH_2$]$Cl_2$, is 1,1/t,t and **II**, [{$Pt(NH_3)_3$}$_2$$H_2N(CH_2)_nNH_2$]$Cl_4$ is 0,0 etc.

Flameless Atomic Absorption Spectroscopy (FAAS)

A Perkin-Elmer 560-AAS instrument with a graphite furnace was used to determine the relative binding (r_b) of platinum complex per given DNA concentration for each condition. The samples were concentrated by complete lyophilization at room temperature using a Savant Speed-Vac Concentrator and then resuspended with 2% nitric acid to half the initial sample volume (500 μl). The samples were incubated at 37°C for 24 hours in order to hydrolyze the DNA. Further dilution of the samples was performed using 2% nitric acid in order to bring the platinum absorbance readings within the range of values obtained using platinum standardizing solutions. Relative binding (r_b) values for each complex were calculated using the platinum concentration, [Pt], determined from the FAAS and the DNA concentration, [DNA], previously obtained by UV/Vis spectroscopy. Thus, ([Pt]/2)/[DNA] = relative binding of complex per nucleotide (r_b).

Interstrand cross-linking

A 5′-terminally labeled 49 bp fragment (1 ng) (See Ref. 12 for sequence) was treated with 10 μM of cis-DDP (Lane 2), [{*trans*-$PtCl(NH_3)_2$}$_2$$H_2N(CH_2)_nNH_2$]$^{2+}$ n = 2, (Lane 3); n = 3, (Lane 4); and n = 4 (Lane 5) at 10 μM in a 10 mM $NaClO_4$, 10 mM Tris pH 7.0 buffer for 1 hour at 37°C. The treated samples were analyzed by electrophoresis on a 7 M urea, 8% polyacrylamide sequencing gel for 1.5 hours. Upon drying, the gel was placed into a Betascope 603 blot analyzer for ^{32}P imaging and quantitation.

RESULTS

The bis(platinum) complexes used in this study are shown in Figure 1. They were chosen to examine the contribution to the conformational change of diamine chain length and the overall charge of the complex in the absence of covalent binding. Consistent with our previous scheme Complexes **Ia** and **Ib** are abbreviated 1,1/t,t, which denotes two monodentate platinum coordination spheres with the chloride leaving groups trans to the diamine bridge. The tetra-amine complexes, **IIa** and **IIb**, are simply abbreviated as 0,0, which indicates that the platinum coordination spheres of these complexes completely lack leaving

Table 1. Binding (r_b) and circular dichroism (CD) spectral parameters for bis(platinum)-DNA complexes[a]

Complex	Dose (μM)	r_b[b]	poly(dG-dC)·poly(dG-dC) λ_{max} (nm)[c]	poly(dG-dC)·poly(dG-dC) λ_{min} (nm)	poly(dG-m^{5}dC).poly(dG-m^{5}dC) r_b	poly(dG-m^{5}dC).poly(dG-m^{5}dC) λ_{max} (nm)	poly(dG-m^{5}dC).poly(dG-m^{5}dC) λ_{min} (nm)
Ia	0	–	275	252	–	275	252
	12.5	0.019					
	25.0	0.025					
	50.0	0.027	274	290			
	100.0	0.51					
Ib	12.5	0.013	275	290	0.010	275	295
	25.0	0.025	270	290	0.024	275	295
	50.0	0.028	267	290			
	100.0	0.059					
IIa	25.0	–	275	296	–	–	–
IIb	5.0	–					
	25.0	–	265	292	–	271	305

[a]See Materials and Methods for details. **I** is $[\{trans\text{-}PtCl(NH_3)_2\}_2H_2N(CH_2)_nNH_2]Cl_2$, n = 2 **Ia**, n = 4 **Ib**. **II** is $[\{Pt(NH_3)_3\}_2H_2N(CH_2)_nNH_2]Cl_4$, n = 2 **IIa**, n = 4 **IIb**.
[b]Average of at least two independent experiments for **Ib**. poly(dG)·poly(dC) gives an r_b of 0.020 with **Ia** and an r_b of 0.017 with **Ib** at a dose of 25 μM.
[c]Intermediate spectra not included for **Ia**. For **Ib** λ_{max} refers to value of positive band appearing with increasing r_b.

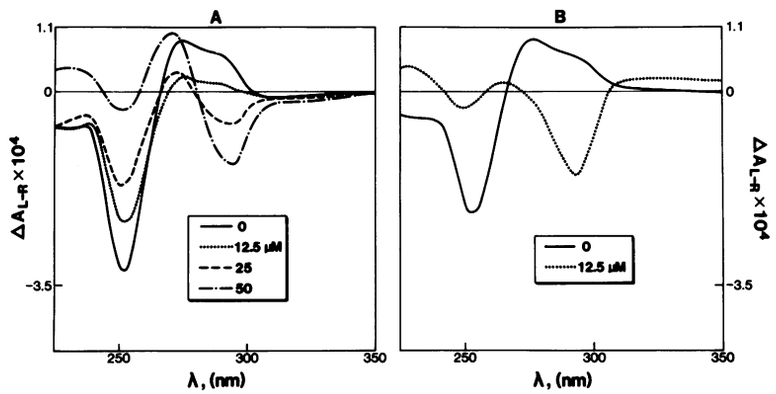


Figure 2. CD spectra of (A) poly(dG-dC)·poly(dG-dC) and (B) poly(dG-m^{5}dC)·poly(dG-m^{5}dC) modified by $[\{trans\text{-}PtCl(NH_3)_2\}_2H_2N(CH_2)_4NH_2]Cl_2$ (Complex **Ib**). The corresponding r_b values are found in Figure 3 and Table 1.

groups such as water or chloride. The DNAs studied were poly(dG-dC)·poly(dG-dC), poly(dG-m^{5}dC)·poly(dG-m^{5}dC) and poly(dG)·poly(dC). Table 1 summarises the binding and spectral data obtained.

Absorbance spectrum of DNA modified by bis(platinum) complexes

The absorbance spectra of all Pt-DNA samples were run to study the change in the spectra upon binding and to check the concentration of DNA after dialysis for any loss due to aggregation. For the cations **IIa** and **IIb** no significant alteration in either band maximum or absorbance was observed in the presence of up to 25 μM of complex. For complexes **Ia** and **Ib**, the absorbance spectra showed features typical of bis(platinum) complex binding to DNA with slight hypochromicity and a red shift of 1–3 nm (12). Only at the highest r_b used (corresponding to 100 μM doses) was significant aggregation observed. This again corresponds with our previous observations (12). Further, the absorbance spectra at low doses did not show any scattering tails beyond 300 nm which might be indicative of the presence of larger particles.

The 'tailing' observed in some of the CD spectra (See individual Figures) at wavelengths longer than 340 nm could be due to particle aggregation which will affect the spectrum (19). The absorbance spectra confirm that no aggregation is seen except at the highest doses used for **Ib**. This 'tailing' of the CD spectra is not consistently observed for all spectra and does not correlate with large absorbance decreases due to possible aggregation. While some aggregation can not be ruled out, the observation of a small amount of absorbance above 340 nm may result from the presence of a number of intermediate species thus complicating the spectra. Future experiments will address this point. The fundamental feature of a clear B→Z transition is not affected by these considerations.

Characterization of the helical distortion induced by $[\{transPtCl(NH_3)_2\}_2H_2N(CH_2)_nNH_2]Cl_2$ (1,1/t,t)

From our initial studies, the conformation of CT DNA modified by the complex **Ib** ($[\{trans\text{-}PtCl(NH_3)_2\}_2H_2N(CH_2)_4NH_2]Cl_2$) was suggestive of a transition from B-form DNA to DNA having 'Z-like' properties (12). As the base specificity of these complexes includes alternating purine (G) pyrimidine (C) sequences, we

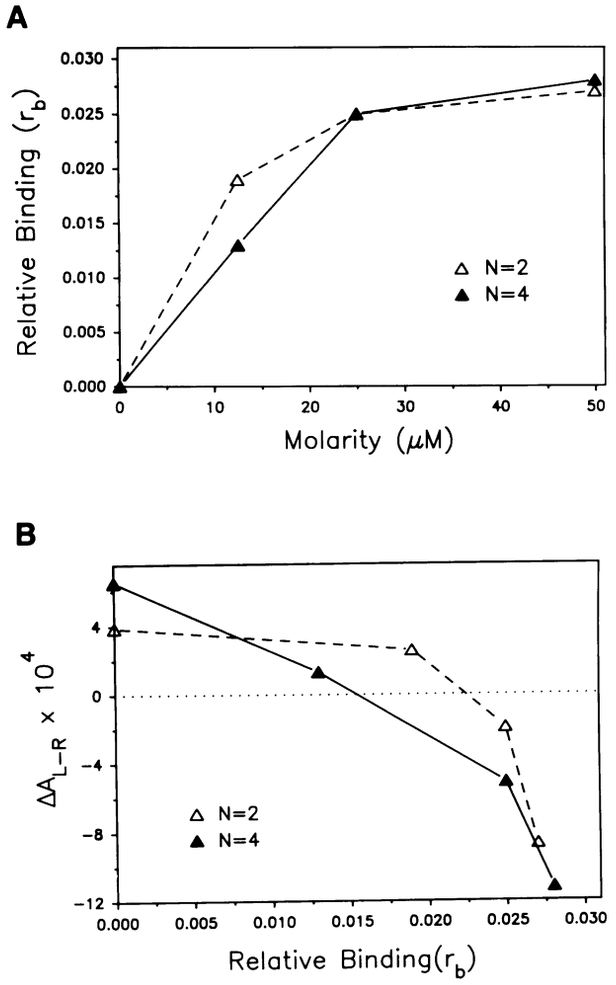


Figure 3. (A) Binding (r_b) as a function of concentration and (B) alteration in absorbance of the CD spectrum at λ_{min} = 290 nm as a function of (r_b) for poly(dG-dC)·poly(dG-dC) modified by [{trans-$PtCl(NH_3)_2$}$_2$$H_2N(CH_2)_nNH_2$]$Cl_2$ (Complexes **Ia**, n = 2, and **Ib**, n = 4).

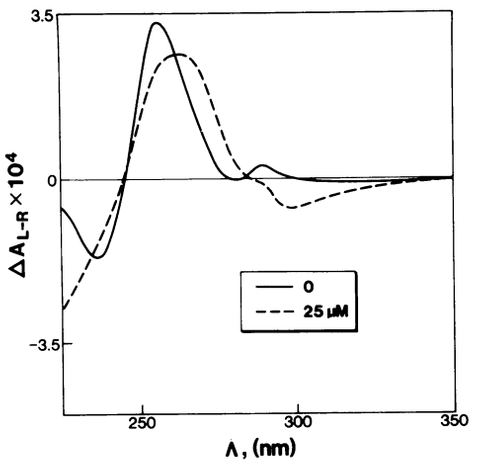


Figure 4. CD spectrum of poly(dG)·poly(dC) modified by [{trans-$PtCl(NH_3)_2$}$_2$$H_2N(CH_2)_4NH_2$]$Cl_2$ (Complex **Ib**).

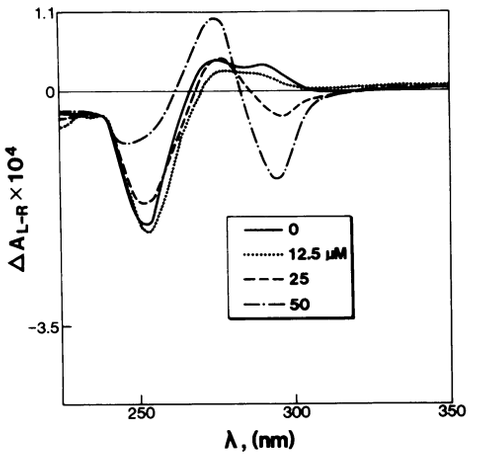


Figure 5. CD spectra of poly(dG-dC)·poly(dG-dC) modified by [{trans-$PtCl(NH_3)_2$}$_2$$H_2N(CH_2)_2NH_2$]$Cl_2$ (Complex **Ia**). The corresponding r_b values are found in Figure 3 and Table 1.

examined their interaction with poly(dG-dC)·poly(dG-dC), which readily adopts the Z conformation (20, 21). Figure 2A shows that Complex **Ib** causes a B→Z transition in poly(dG-dC)·poly(dG-dC) in a dose dependent manner. We have chosen to describe the 'degree of Z-character' of each sample by plotting the molar ellipticity at λ_{min} = 290 nm as a function of Pt bound to the polynucleotide, Figure 3. Increasing positive molar ellipticity values indicate increased 'B-character' while decreasing negative values reflect more 'Z-character' in the conformation of the DNA (22). We define 50% of the DNA to be in the Z-form when ΔA_{L-R} = 0. Using this criterion, the 50% B→Z transition occurred at r_b 0.016 for Complex **Ib**.

The energy of activation between the B- and Z-form of poly(dG-m^{5}dC)·poly(dG-m^{5}dC) is significantly lower than that for the unmethylated poly(dG-dC)·poly(dG-dC) (23). Accordingly, Figure 2B shows that, as expected, Complex **Ib** induces the B→Z transition in this DNA at a lower concentration. The transition appeared to be complete at a dose of 12.5 μM, corresponding to an r_b of 0.013. To ascertain whether a sequence of alternating purines and pyrimidines is necessary for Z-DNA formation, the changes in poly(dG)·poly(dC) upon complex binding were examined. No B→Z transition was observed at equivalent binding, Figure 4, or even when using an order of magnitude higher concentration (100 μm) of **Ib** in the assay (Data not shown).

Minimum structural features required for the B→Z transition

Bis(platinum) complexes with monodentate coordination spheres carry a formal 2+ charge, and possess hydrogen-bonding amine ligands, a hydrophobic (diamine linker) region as well as two charged platinum moieties capable of covalent DNA-binding. Any of these features alone could account for the B→Z induction. We sought to assess the contribution of chain length on the conformational switch by synthesis of the n = 2 analogue, **Ia** (1,1/t,t n = 2). Figure 5 shows that the binding of **Ia** also results in a Z-like spectrum in a dose dependent manner very like that of **Ib** (n = 4), with the 50% B→Z transition in poly(dG-dC)·poly(dG-dC) occurring at an r_b = 0.023.

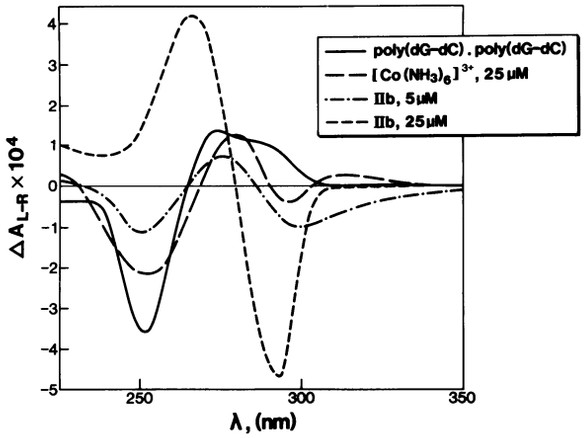


Figure 6. CD spectra of poly(dG-dC)·poly(dG-dC) in the presence of 25 μM concentrations of the cations $[Co(NH_3)_6]^{3+}$, $[Pt(NH_3)_4]^{2+}$ and $[\{Pt(NH_3)_3\}_2H_2N(CH_2)_nNH_2]Cl_4$ (Complexes **IIa**, n = 2 and **IIb**, n = 4).

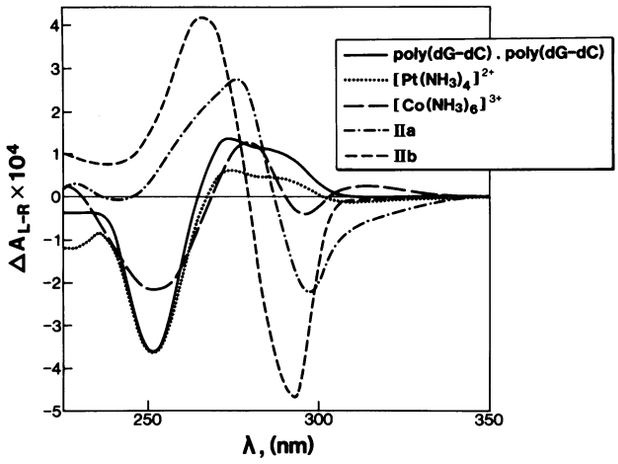


Figure 7. CD spectra of poly(dG-dC)·poly(dG-dC) in the presence of $[Co(NH_3)_6]^{3+}$, 25 μM, and $[\{Pt(NH_3)_3\}_2H_2N(CH_2)_4NH_2]Cl_4$ (Complex **IIb**), 5 and 25 μM.

The CD spectra of poly(dG-dC)·poly(dG-dC) modified by the two 1,1/t,t complexes are not, however, identical. The spectral parameters are summarised in Table 1. In the n = 2 case the concentration dependence shows an apparent isostrophic point, Figure 5. Further, the λ_{max} of the positive band in the CD spectrum at 275 nm is essentially invariant. In contrast, the maxima for the n = 4 1,1/t,t derivative, **Ib**, suffer progressive blue shifts with increasing Pt bound. At a dose of 50 μM (r_b = 0. 028) the λ_{max} of the positive band is at 267 nm. A similar blue shift was observed for the spectrum of CT DNA modified by **Ib** (12). For both complexes, the minimum of the negative band is centered at 290 nm.

In order to conclude that both the n = 4 and n = 2 forms of the 1,1/t,t complex could induce a B→Z transition with similar efficiencies, it was important to determine whether they had the same reactivity to DNA. The amount of complex bound to the DNA per given dose was determined by flameless atomic absorption spectroscopy (See Materials and Methods). Figure 4 and Table 1 show that the n = 4 and n = 2 complexes have very similar reactivities toward DNA as they both form a comparable number of DNA adducts at any given concentration. An interesting characteristic of this binding profile is that at lower concentrations (less than 25 μM or approximately 0.025 r_b) the formation of DNA adducts is linear, while at greater concentrations the binding of additional bis(platinum) complexes to DNA appears to level off. This trend is similar to the relative binding (r_b) pattern for bis(platinum) complexes observed using CT DNA (12). The conformational change does not appear to affect bis(platinum) complex binding as the final r_b for poly(dG-m^5dC)·poly(dG-m^5dC) is also similar to the unmethylated polynucleotide at the same doses, Table 1. In contrast, the binding profile for cis-DDP is linear throughout the r_b range 0−0.2 for both poly(dG-dC)·poly(dG-dC) and poly(dG-m^5dC)·poly(dG-m^5dC) (24). The relative binding of cis-DDP appears to be unaffected by whether these polynucleotides are in the B or Z-form (23, 25).

Contribution to Z-DNA induction by charge effects

Finding that diamine chain length variation did not appreciably affect the Z-DNA inducing properties of the 1,1/t,t complexes we looked at the contribution of charge effects independent of covalent binding. Polyvalent cations such as $[Co(NH_3)_6]^{3+}$ ions are very effective inducers of the B→Z transition (22, 26). The tetra-amine analogues of **Ia** and **Ib**, $[\{Pt(NH_3)_3\}_2H_2N(CH_2)_nNH_2]Cl_4$ (**IIa**, n = 2 and **IIb**, n = 4) were prepared and their interaction studied. The monomeric species $[Co(NH_3)_6]^{3+}$ and $[Pt(NH_3)_4]^{2+}$ were also studied to examine effects of size and charge. The B→Z transition is highly dependent on the $[Na^+]$ present (22, 25). Therefore, for the polyvalent non-binding cations, we used low $[Na^+]$ conditions of 10 mM {poly(dG-dC)·poly(dG-dC)} or 1.5 mM {poly(dG-m^5dC)·poly(dG-m^5dC)}. At these $[Na^+]$ levels the B-form of the polynucleotide is preferred (19).

Figure 6 shows that the bis(platinum) tetra-amine complexes are remarkably efficient at inducing the B→Z conformation in poly(dG-dC)·poly(dG-dC). Thus, covalent binding is not a prerequisite for the Z-DNA inducing behavior of bis(platinum) complexes. The concentration of cation used, 25 μM, corresponds to an 'r_b' of 0. 5 with 1 complex per 2 phosphate charges in each case. The relative concentration of $[Co(NH_3)_6]^{3+}$ (20 μM) at the end point of the B→Z transition in poly(dG-dC)·poly(dG-dC) has been calculated as 2 positive charges per phosphate in 50 mM NaCl, 5 mM Tris, pH 8.0 (22). Under our slightly different conditions, the $[Co(NH_3)_6]^{3+}$ cation at 25 μM did not achieve the 50% transition although the negative band at 290 nm is clearly beginning to appear, Figure 6. In contrast, both bis(platinum) complexes **IIa** and **IIb** induce completely the Z-form at this concentration. Similar to the 1,1/t,t complexes, the spectra of poly(dG- dC)·poly(dG-dC) modified by **IIa** and **IIb** are not identical. For n = 2 the λ_{min} of the negative band is centered at 296 nm while the positive band is centered at 275 nm. In the case of the n = 4 complex the negative band is centered at 292 nm but the positive band is again blue shifted to 265 nm. These trends are similar to those observed for **Ia** and **Ib** (See above). Note that the pair of spectra for **Ia** and **IIa** (both n = 2) or **Ib** and **IIb** (both n = 4) are again similar but not superimposable. The C.D. spectrum of poly(dG- dC)·poly(dG-dC) modified by **IIb** is dependent on concentration, Figure 7. A rigorous comparison of the Z-DNA inducing ability of the hexamminecobalt and bis(Pt) cations would have to take into

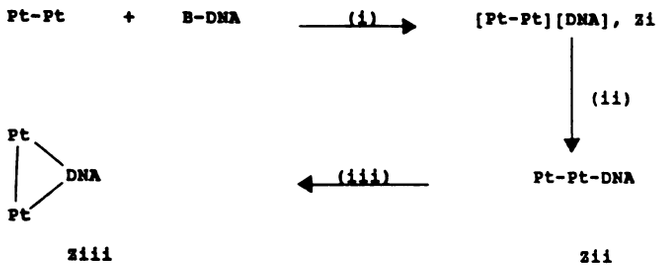
account their relative affinities (27, 28) but it is clear from Figure 7 that the concentration of **IIb** required to produce similar spectral changes to those of $[Co(NH_3)_6]^{3+}$ is approximately 20% that of the Co species. The broadness of the CD bands in poly(dG-dC)·poly(dG-dC) modified by the polyvalent cations appears typical (22, 25).

As expected, **IIb** is also very effective at inducing the B→Z transition in poly(dG-m^{5}dC)·poly(dG-m^{5}dC) at a Pt complex:phosphate ratio of 1:2 (spectra not shown). The monomeric platinum tetra-amine analog, $[Pt(NH_3)_4]^{2+}$, completely lacked any B→Z transitional activity with poly(dG-dC)·poly(dG-dC) (Figure 6) and only a slight hypochromic effect in the positive band is apparent even up to 100 μM (Data not shown).

DISCUSSION

In this study, we have shown that poly(dG-dC)·poly(dG-dC) modified by bis(platinum) complexes with monodentate coordination spheres, $[\{trans\text{-}PtCl(NH_3)_2\}_2H_2N(CH_2)_nNH_2]Cl_2$ (**I**,1,1/t,t) adopts a Z-like structure. Covalent binding is not a prerequisite for this conformational switch because the positively charged tetra-amine bis(platinum) complexes, $[\{Pt(NH_3)_3\}_2H_2N(CH_2)_nNH_2]Cl_4$ (**II**), also effect this change at low concentrations. In fact, these latter agents are even more efficient than the well known $[Co(NH_3)_6]^{3+}$ cation in stabilizing the left-handed helix. The interesting situation arises that the final 'steady-state' spectrum of DNA modified by covalent binding of **Ia** and **Ib** is in fact a reflection of changes occurring prior to covalent binding of both Pt atoms.

Mechanism of Z-DNA stabilization by bis(platinum) complexes. The interaction of bis(platinum) complexes with monodentate coordination spheres (**I**,1,1/t,t) with DNA may be presented:



Three distinct steps may be defined:

(i) The complex electrostatically associates with the polynucleotide through the NH_3 ligands and the negatively charged phosphate backbone. In the case of the tetra-amine complexes, **II**, this produces a conformational change Zi.

(ii) One platinum atom of the complex covalently binds to the N7 of a guanine base, producing a further change to Zii.

(iii) The second platinum atom attaches in a second step to a further guanine N7 position forming a cross-link. The conformation induced may now be equivalent to Ziii.

Two major factors influencing the B→Z equilibrium are ionic changes in solution and covalent modification (20). The electrostatic interactions between bis(platinum) tetra-amine species and poly(dG-dC)·poly(dG-dC) stabilize the Z-form (Zi in scheme above). In the case of 1,1/t,t complexes both ionic effects and covalent modification effects will contribute to the overall conformational changes. Since the rate-determining step for Pt-DNA binding is chloride hydrolysis the reactive aqua form

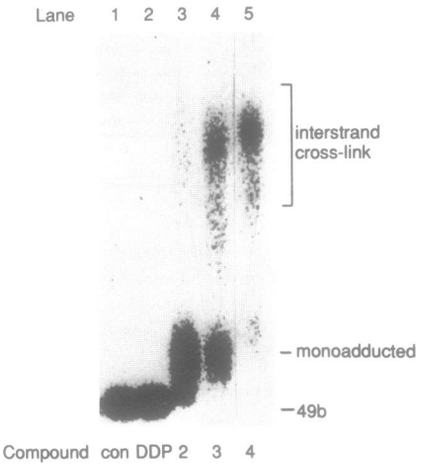


Figure 8. Interstrand cross-link formation by cis-DDP (Lane 2) and $[\{trans\text{-}PtCl(NH_3)_2\}_2H_2N(CH_2)_nNH_2]Cl_2$ (n = 2, Lane 3; n = 3, Lane 4 and n = 4, Lane 5). See Materials and Methods and Ref. 12 for details.

will be $[\{trans\text{-}Pt(H_2O)(NH_3)_2\}_2H_2N(CH_2)_nNH_2]^{4+}$ (29). The H-bonding effects of this species are unlikely to be greatly different to the tetra-amines **II** and we can expect similar efficiency in B→Z changes. Covalent binding will further alter the conformation.

The further question now arises of whether binding of both Pt atoms in the 1,1/t,t complexes is necessary for stabilizing the Z-form. The r_b values for n = 2 and n = 4 are very similar, Figure 3, but interstrand cross-linking in $[\{trans\text{-}PtCl(NH_3)_2\}_2H_2N(CH_2)_nNH_2]^{2+}$ is significantly reduced in the order n = 4 > 3 > 2, Figure 8. These results would argue that the binding of both Pt atoms is also not a necessary requirement for Z-DNA formation. This discrete Z-form (Zii) will be altered further upon the subsequent formation of the second covalent bond (Ziii). The spectral differences between complexes of the same chain length (**Ia/IIa** and **Ib/IIb**) support this step-wise interpretation of the bis(Pt-DNA) interaction.

The N7-N7 distance between the guanines in a d(GC) base pair in the $(GpC)_n$ sequence varies from 7.2 to 8.2 Å in the B-form and alternates between 8.4 and 13.68 Å in the Z-form (30). It is intriguing to consider that the reason for the reduced interstrand cross-linking for the n = 2 derivative (**Ia**) is that the conformational changes upon binding of the first Pt center makes binding of the second center to an adjacent guanine sterically unfavorable.

In summary, the bis(platinum) complexes produce a range of Z-form conformers, dependent on the exact nature of the Pt-DNA interaction. Covalent binding is not an absolute necessity for the Z-DNA induction but interstrand cross-linking may be very useful in 'locking' the Z-conformation (31). This behavior is in contrast to that of monomeric complexes. The B→Z transition is facilitated by [PtCl(dien)]Cl but Pt-base binding must occur (21, 24). In contrast, cis-DDP actually stabilizes B-form DNA (21, 23, 24).

Biological consequences of Z-DNA stabilization. There are distinct areas within eukaryotic genomes that contain relatively long stretches of alternating purine-pyrimidine $(GpC)_n$ (so-called 'CpG islands') or $(GpT)_n$ sequences. These sequences are

almost always located in the 5′ promotor region of the 'housekeeping-genes' and to a lesser extent can also be found in tissue specific gene promotors (32, 33, 34). Since the $(GpT)_n$ motif can also form Z-DNA (35, 36) it is not surprising that $(GpT)_n$-containing genomic DNA sequences have been found to adopt the Z conformation in *E.coli* at physiological superhelical density (33). The exact biological consequences of Z-DNA are still a subject of speculation (37) but the induction of Z-DNA has been found to inhibit transcription in an *in vitro* reaction (38).

The presence of CpG rich sequences in CT DNA would provide a good target for bis(platinum) complexes. The facile B→Z transformation in poly(dG-dC)·poly(dG-dC) supports the contention that localized Z-form regions could account for the helical distortions induced on CT DNA by **Ib**, and consequently the unusual CD spectrum observed (12). In cells, this conformational change could contribute to the cytotoxic effects of bis(platinum) complexes. The fact that Z-DNA is stabilized by bis(platinum) complexes with monodentate coordination spheres whereas B-DNA is stabilized by cis-DDP represents a major difference in how these two sets of compounds alter DNA conformation. This unique feature of these bis(platinum) complexes could be exploited both as probes for DNA conformation and in developing antitumor agents acting by different mechanisms to the presently used drugs.

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