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Conformation of Poly(dG-dC)·Poly(dG-dC) and Poly(dA-dT)·Poly(dA-dT) Interacting with the Antitumor Drug *cis*-$[Pt(NH_3)_2]Cl_2$

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The interaction of cis*-dichlorodiammine platinum-(II) with poly(dG-dC)·poly(dG-dC) and poly(dA-dT)·poly(dA-dT) was studied by circular dichroism. Significant conformational changes were induced in both alternating polymers: in the case of poly(dG-dC)·poly(dG-dC) the spectra were not conclusive in terms of a well defined conformation, even if the presence of left-handed helices could be suggested. For poly(dA-dT)·poly(dA-dT) the data were interpreted in terms of a dimer-helix → single hairpin helix transition induced by the metal. The results obtained are discussed with reference to the antitumor activity of the drug.*

Introduction

It is widely believed that the primary cellular target of the antitumor drug *cis*-dichlorodiammine platinum(II) (*cis*-platinum) is DNA. A variety of *in vitro* studies [1–5] suggested that *cis*-platinum binds covalently to bases in DNA, the order of binding affinity being guanine > adenine > cytosine ≫ thymine, with a strong kinetic preference for the N-7 position of guanine. Several models of bifunctional binding of *cis*-platinum to DNA have been proposed such as intrabase chelation at the O-6 and N-7 positions of guanine [2, 6, 7], interstrand crosslinking between the N-7 positions of guanines in opposite strands [8, 9] and/or intrastrand crosslinks between two guanines in the same strand [9–11]. Very recently, Brouwer *et al.* [12] have reported convincing evidence for binding to two non-adjacent guanines in a GAG or GCG sequence on the same strand.

As far as the variations induced in DNA are concerned, more or less localized conformational changes (depending on the Pt/P ratios used) have been demonstrated by absorbance–temperature profiles, circular dichroism and Raman spectroscopy [3, 4, 9, 13–16]. However, the precise nature of these structural changes remains to be verified together with their relevance in connection to the antitumor activity of *cis*-platinum. Accordingly, we decided to investigate by circular dichroism the conformational changes induced by *cis*-platinum on simple alternating copolymers, namely poly(dG-dC)·poly(dG-dC) and poly(dA-dT)·poly(dA-dT). Some data on poly(dG-dC)·poly(dG-dC), reported by different authors [17, 18], gave different spectral results.

Experimental

Poly(dG-dC)·poly(dG-dC) and poly(dA-dT)·poly(dA-dT) were purchased from P. L. Biochemicals, Inc., Milwaukee, U.S.A.; *cis*-platinum was a product of Strem Chemicals, Inc., Newburyport, U.S.A. Circular dichroism(CD) spectra were recorded either on a Cary model 61 or on Jasco 500A dichrographs. Quartz cells were used with 0.5, 0.1 and 0.05 cm optical paths. Samples of copolymers were examined at a concentration of about 10^{-3} *M* on a nucleotide base. The data are expressed in terms of $[\theta]$ the mean residue molecular ellipticity in units of degree cm^2 $dmol^{-1}$. If not otherwise stated, the spectra were measured at 20 °C. The concentration of the solution of copolymers was determined spectrophotometrically at the wavelength of the maximum of absorption, using the following values of ϵ(mol phosphorous P cm^{-1} l): poly(dG-dC)·poly(dG-dC) 8,400 (λ_{max} 254 nm); poly(dA-dT)·poly(dA-dT) 6,600 (λ_{max} 262 nm).

Results

In Fig. 1 the CD spectra of poly(dG-dC)·poly(dG-dC) in 5 m*M* NaCl at different Pt/P input ratios are reported. The major spectral variations are seen at Pt/P = 0.3–0.5; under these conditions the longer wavelength positive band inverts its sign and the whole spectrum to about 250 nm appears to assume an inverted semi-conservative character. This behavior is somewhat similar to that reported by Malfoy *et al.* [17] but differs from that observed by Ushay *et al.* [18]. The spectra at higher Pt/P ratios are qualitatively similar in the region of longer wavelength to those obtained by Pohl and Jovin [19] at high NaCl

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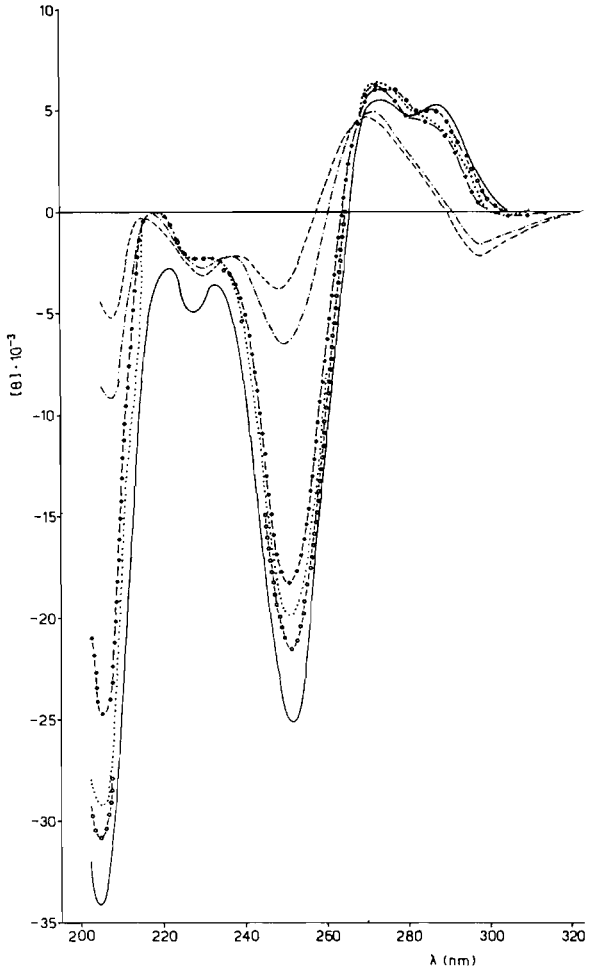

Fig. 1. CD spectra of poly(dG-dC)·poly(dG-dC) in the presence of *cis*-platinum at different Pt/P input ratios (r): (——) r = 0; (—○—) r = 0.05; (·····) r = 0.08; (—*—) r = 0.1; (— · —) r = 0.3; (- - - -) r = 0.5. Solvent 5 m*M* NaCl.

concentration; thereafter similar CD changes were observed on increasing the concentration of $CsSO_4$ [20, 21], in the presence of Co^{2+} or Mn^{2+} together with ethylene glycol or ethanol [22], in trifluoroethanol–water mixtures [23], after modification at the C-8 position of guanine by N-acetoxy-N-acetyl-2-aminofluorene [24], after methylation at the N-7 position of guanine through the use of dimethyl sulfate [25], and after interaction with mitomycin C [26]. It is generally agreed that the low-salt form is in the B-DNA conformation, while the high-salt form is in the so called Z-DNA conformation [27–30], *i.e.* a left-handed array. Figure 2 shows that the transition is rather cooperative as judged by CD: this is what would be expected for a B → Z conformational transition [19, 20]. However, the spectrum does not exactly equal that characteristic of the Z-form.

In order to get more insight into this problem we studied the effects of *cis*-platinum on an authentic B → Z transition of poly(dG-dC)·poly(dG-dC).

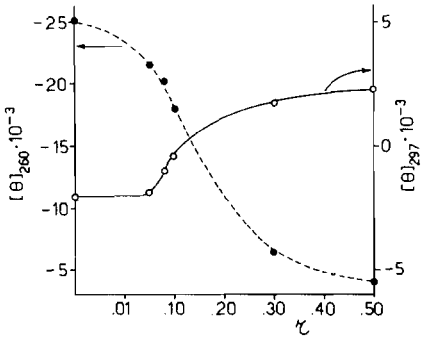

Fig. 2. Variation of the ellipticity of the longer wavelength bands as a function of Pt/P input ratio (r) for poly(dG-dC)·poly(dG-dC) interacting with *cis*-platinum. Solvent as in Fig. 1.

Figure 3 shows the transition between the B and Z forms of the polynucleotide induced by trifluoroethanol. Essentially, the results are similar to those of Ivanov and Minyat [23]: it is evident from the isodichroic point at ~272 nm that only two conformations are present in the absence of *cis*-platinum. However, this is no longer true for the spectrum of platinated poly(dG-dC)·poly(dG-dC); actually, the curve is rather broad with some wavelength shifts at both the negative and positive extrema. It seems therefore that the conformational change induced by the binding of the metal is not simply a B → Z transition. This is further confirmed by the spectra shown in Fig. 4. It is evident that *cis*-platinum is not able to shift to the right the B → Z transition in trifluoroethanol. Then what do these spectral changes mean? In view of the fact that the Z form can be reversibly transformed into the B and A forms [23], the possibility exists that the spectra in the presence of *cis*-platinum are those resulting from the mixture of three conformers, namely the A, B and Z forms. As a matter of fact, the platinum complex is able to stabilize the A form under appropriate conditions. Figure 5 is unequivocal in this respect. Actually, the figure shows the circular dichroism spectra of the nucleic acid in 60% ethanol in the presence and absence of *cis*-platinum. At this percentage of ethanol poly(dG-dC)·poly(dG-dC) is undergoing an order-to-order transition from the Z-form to the A-form [31]. The spectrum is indeed characterized by a small negative band at 295 nm and by a large positive band at ~273 nm suggesting a strong contribution from the A-form. On adding $\sim 10^{-3}$ *M* CsI to the ethanolic solution the positive band decreases considerably, probably indicating an A to B transition. In fact, Ivanov *et al.* [32] showed that Cs^+ ions stabilize the B form of DNA with respect to the A form. On the contrary, *cis*-platinum does stabilize the A-form, as shown by the increase of the large positive band in the spectrum of Fig. 5. Figure 6 reports the spectra of poly(dA-dT)·poly(dA-dT) interacting with *cis*-

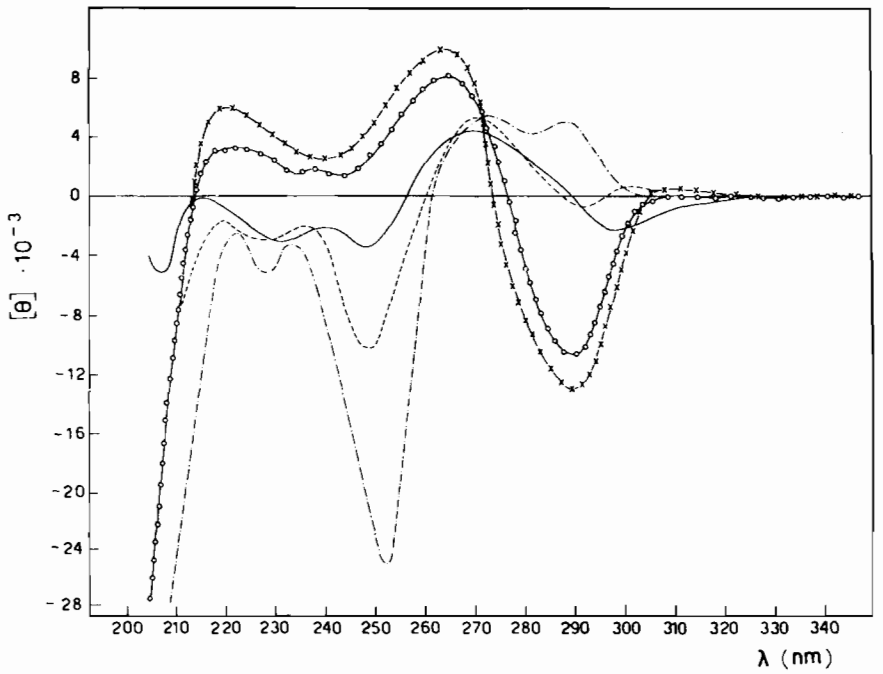

Fig. 3. CD spectra of poly(dG-dC)·poly(dG-dC) in trifluoroethanol–water mixtures. (—·—), water; (- - -), 51% trifluoroethanol; (–○–), 60% trifluoroethanol; (–×–), 64% trifluoroethanol. For comparison the curve of the polynucleotide in water plus *cis*-platinum (Pt/P = 0.5) is also reported (——).

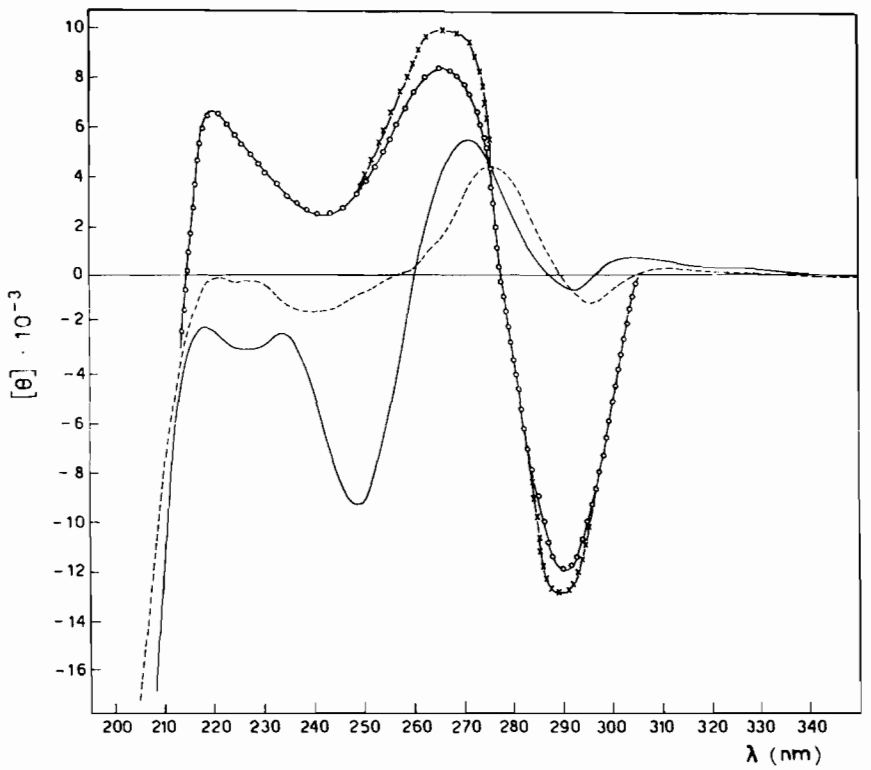

Fig. 4. CD spectra of poly(dG-dC)·poly(dG-dC) in 51% trifluoroethanol in the absence (——) and in the presence of *cis*-platinum (Pt/P = 0.5) (- - - -), and in 64% trifluoroethanol in the absence (–×–) and in the presence of *cis*-platinum (Pt/P = 0.5) (–○–).

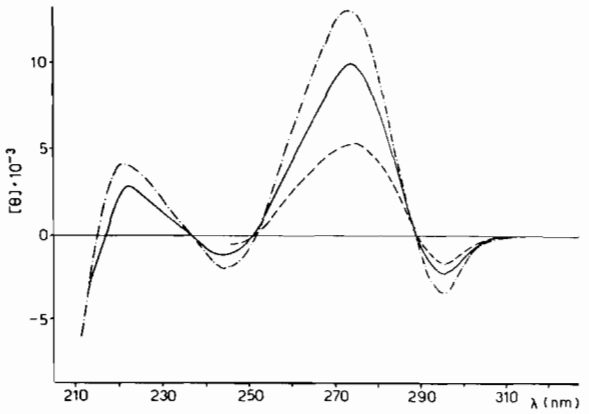

Fig. 5. CD spectra of poly(dG-dC)·poly(dG-dC) in 60% ethanol in 5 m*M* NaCl (——); plus 3 m*M* CsI (- - -); plus *cis*-platinum (Pt/P = 0.5) (—·—).

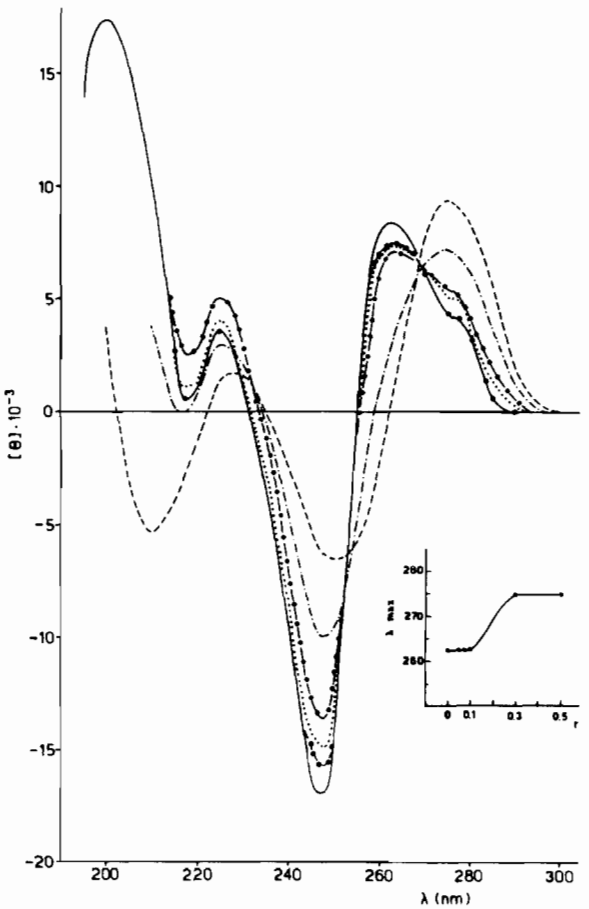

Fig. 6. CD spectra of poly(dA-dT)·poly(dA-dT) in the presence of *cis*-platinum at different Pt/P input ratios. Conditions and symbols as in Fig. 1. Inset: variations of the λ_{max} of the longer wavelength positive band as a function of Pt/P input ratio (r).

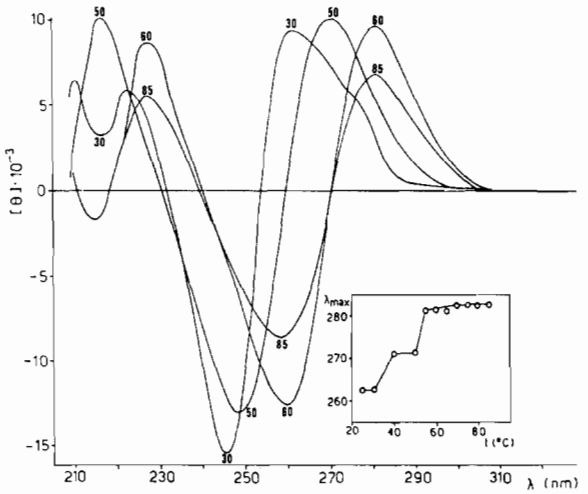

Fig. 7. CD spectra of poly(dA-dT)·poly(dA-dT) at the indicated temperatures. Solvent as in Fig. 1. Inset: variation of the λ_{max} of the longer wavelength positive band as a function of the temperature.

platinum. Even in this case the interaction with the platinum derivative induces significant spectral changes: in particular, the positive band at longer wavelengths is red-shifted (about 13 nm) on increasing the Pt/P ratio (isodichroic point at about 270 nm), without a straightforward change in the intensity, while the negative band is greatly reduced and only slightly red-shifted. These CD changes mimic well those obtained by heating the polymer at low temperatures (Fig. 7). As a matter of fact the heat denaturation of the polymer consists of two well defined transitions. According to Baldwin [33], the first one is related to a transition, in the submelting temperature range, from a dimer helix to an intramolecular hairpin helix which cooperatively melts at higher temperatures. Thus, it is tempting to suggest that the binding of *cis*-platinum induces strand separation in poly(dA-dT)·poly(dA-dT) with subsequent rearrangement of the chains. Similar behaviour has been observed recently for a self-complementary deoxydodecanucleotide [34].

Discussion

The results of our study clearly indicate that *cis*-platinum is able to induce conformational changes in both alternating polymers. Of course, the fact that *cis*-platinum binds significantly to poly(dA-dT)·poly(dA-dT) does not imply the same effect on DNA. As mentioned, there is a strong kinetic preference for guanine as fixation site, hence the initial reaction with DNA, especially at low Pt/P ratios, should occur primarily at guanine. However, an additional attack on adenine cannot be ruled out. The partial denaturation

induced in poly(dA-dT)·poly(dA-dT) may also be of interest in connection with the role played by *cis*-platinum *in vivo*. Actually, it is not unreasonable that in DNAs particularly rich in A-T a local denaturation at these sequences may occur.

Although our data do not allow the precise identification of the conformation induced in poly(dG-dC)·poly(dG-dC), it is possible to suggest the presence of regions of left-handed helices, not necessarily the canonical Z-form, belonging to the family of the L-duplexes according to Goupta *et al.* [35]. One spectrum similar to those found by us was reported in the literature, referring to the so called V-DNA form [36]. The spectrum was not related to a specific, unequivocal structure, although Stettler *et al.* [36] proposed a mixture of both right-handed and left-handed helices for the V-DNA form. In addition, some regions in the A conformation could also be present within the right-handed helices together with the more usual B form. Finally, it should be noted that den Hartog *et al.* [37] have shown by NMR that Pt chelation by the dinucleotide dG-p-dG induces a C2′-endo to C3′-endo puckering change of one deoxyribose. While the possibility of the same kind of binding in a sequence -dG-p-dC-p-dG- (as in our case) remains to be demonstrated, we emphasize that the inversion C2′-endo → C3′-endo occurs, among other conformational changes, at the guanine nucleotide level in the Z-form.

Taken as a whole, our data, although not conclusive, are suggestive of a complex pattern as far as the structural perturbation of DNA, induced by *cis*-platinum, is concerned. Future work on nucleic acid fragments of known sequences seems important to gain greater insight into this problem.

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