A Study of Interactions of Platinum (II) Compounds with DNA by Means of CD Spectra of Solutions and Liquid Crystalline Microphases of DNA

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Abstract. The optical properties of the DNA complexes with divalent platinum compounds of the *cis*-diamine type differing both in the nature of anionic and neutral ligands and in the spatial arrangement about the platinum atom were studied. The platinum compounds *cis*-$[Pt(NH_3)_2Cl_2]$, $[Pt(en)Cl_2]$, [Pt(tetrameen)Cl_2], *cis*-$[Pt(NH_3)_2NO_2Cl]$, and *cis*-$[PtNH_3(Bz)Cl_2]$ at small values of r (r is the molar ratio of a platinum compound to DNA nucleotides in the reaction mixture) were found to induce an increase in the amplitude of the positive band in the circular dichroic (CD) spectrum of linear DNA. All the compounds listed except *cis*-$[Pt(NH_3)_2NO_2Cl]$ caused a sharp decrease of the amplitude of the negative band in the CD spectrum of a liquid crystalline microphase of DNA formed in solution in the presence of poly(ethylene glycol). All these platinum compounds (except [Pt(tetrameen)Cl_2]) exhibit biological (antimitotic, antitumour, etc.) activity. The platinum compounds *trans*-$[Pt(NH_3)_2Cl_2]$, *trans*-$[Pt(NH_3)_2NO_2Cl]$, *cis*-$[PtNH_3PyCl_2]$, *cis*-$[Pt(NH_3)_2(NO_2)_2]$, and $[Pt(NH_3)_3Cl]Cl$ exhibiting a low (if any) biological activity, either induced a decrease of the amplitude of the positive band in the CD spectrum of linear DNA, or did not affect the CD spectrum at all. The effect of these platinum compounds on the CD spectrum of the liquid crystalline microphase of DNA was either weak or absent. It is assumed that the specific biological action of platinum compounds of the *cis*-diamine type is determined by the polydentate binding to DNA: in addition to the *cis*-bidentate covalent binding of platinum to DNA nitrogen bases, a hydrogen bond formation between the DNA and *cis*-amino ligands occurs by means of protons at nitrogen atoms.

Key words: Linear DNA — Liquid crystalline microphase of DNA — Platinum (II) compounds — Circular dichroism spectrum — Polydentate binding

Introduction

The fact that some *cis*-diamine type platinum compounds possess antitumour activity indicates their potential importance (Connors and Roberts 1974; Prestayko et al. 1980). Both *in vivo* and *in vitro* experiments show that the biological activity of platinum compounds is related to their interactions with DNA. The formation of a complex of DNA with platinum compounds is due to the replacement of anionic labile ligands in platinum compounds and the formation of a covalent bond between platinum and DNA nitrogen bases (Connors and Roberts 1974; Macquet and Butour 1978b). The initial site of attachment is thought to be N(7) of guanine bases (Macquet and Theophanides 1975) and the presence of biological activity only with the *cis* isomers but not with their *trans* analogues is attributed to the ability of *cis* compounds to chelate between N(7) and O(6) of the same guanine or between N(7) of two guanine residues (Macquet and Butour 1978a). Data indicate, however, that a necessary structural requirement for biological activity is the presence of two *cis*-amino ligands containing protons at nitrogen atoms capable of hydrogen bond formation (Ivanov et al. 1981a).

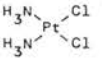
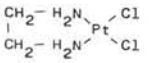
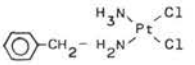
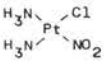
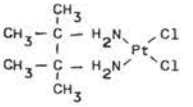
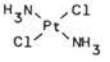
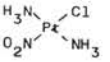
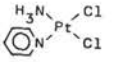
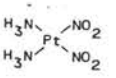
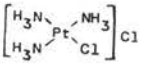
The present study examines the interaction of DNA with a series of platinum compounds that have varying numbers of labile anionic ligands and of neutral ligands of different nature. We employed the method of circular dichroism (CD) for the investigation of both linear DNA molecules and liquid crystalline microphases formed from DNA molecules in water - salt solutions in the presence of poly(ethylene glycol) (PEG). Characteristic changes in the CD spectra of linear DNAs incubated with platinum compounds yielded information concerning the type of interaction (Macquet and Butour 1978a, b; Srivastava et al. 1978; Brabec et al. 1984; Kleinwächter and Rau 1984). The use of the CD spectra of liquid crystalline microphases of DNA was based on a previous observation that the amplitude of the negative band in the CD spectrum of a liquid crystalline microphase diminishes upon perturbation of the secondary structure of the microphase-forming DNA molecules (Evdokimov et al. 1975; Akimenko et al. 1977). Preliminary data on the CD spectra of liquid crystalline microphases of DNA complexes with *cis*- and *trans*-dichlorodiammineplatinum (II) have been published elsewhere (Akimenko et al. 1983).

Materials and Methods

Low-molecular-weight ($\leq 1 \times 10^6$) salmon sperm DNA (41 mole % G.C) (SKTB BAV, Novosibirsk) was obtained by ultrasonic depolymerization of high-molecular-weight DNA (4 °C; 50 s; 22 kHz; ultrasound generator UZDN-2T, USSR). The molecular mass of DNA was estimated by electrophoresis in agarose gel. The DNA concentration was determined from the absorbance of solutions ($\lambda = 260$ nm, $\varepsilon_p = 6600$ l . mol^{-1} cm^{-1}).

Platinum (II) compounds (Table 1) were synthesized and characterized at the Institute of General

Table 1. Names and formulae of the platinum compounds used in the present study

Platinum compounds	Chemical structure
I *cis*-dichlorodiammineplatinum(II) *cis*-[$Pt(NH_3)_2Cl_2$]	
II dichloroethylenediamineplatinum(II) [$Pt(en)Cl_2$]	
III *cis*-dichloroamine(benzylamine)-platinum(II) *cis*-[$PtNH_3(Bz)Cl_2$]	
IV *cis*-chloronitrodiammineplatinum(II) *cis*-[$Pt(NH_3)_2NO_2Cl$]	
V dichloro($\alpha,\alpha,\alpha',\alpha'$-tetramethylethylene-diamine)platinum(II) [$Pt(tetrameen)Cl_2$]	
VI *trans*-dichlorodiammineplatinum(II) *trans*-[$Pt(NH_3)_2Cl_2$]	
VII *trans*-chloronitrodiammineplatinum(II) *trans*-[$Pt(NH_3)_2NO_2Cl$]	
VIII *cis*-dichloroamminepyridineplatinum(II) *cis*-[$PtNH_3PyCl_2$]	
IX *cis*-dinitrodiammineplatinum(II) *cis*-[$Pt(NH_3)_2(NO_2)_2$]	
X chlorotriammineplatinum(II) chloride [$Pt(NH_3)_3Cl$]Cl	

and Inorganic Chemistry of the USSR Academy of Sciences according to procedures described elsewhere (Chernyaev 1964; Ivanov et al. 1981a).

Solutions of DNA, PEG (mol. mass 4000; Loba Chemie, Austria), and platinum compounds were prepared using 0.3 mol . l^{-1} $NaClO_4$ (pH 5.8) as a solvent. The solutions of platinum compounds were taken into experiment not earlier than 24 h after the preparation (Kleinwächter 1972). The concentration of platinum in the initial solutions was estimated by the plasma-induced emission spectroscopy using a Jobin-Yvon 38 emission spectrometer (France) (Baluda et al. 1980).

The reaction of DNA with platinum compounds was carried out by mixing the appropriate solutions and storing the mixture at 37 °C in the dark for 3 days. The concentration of DNA in the mixture was constant ($\sim 4 \times 10^{-5}$ mol . l^{-1} nucleotides), the concentration of platinum compounds being chosen so that the molar ratio of platinum to DNA nucleotides in solution (r) varied from 0.005 to 1.0.

The liquid crystalline microphase was formed by vigorously mixing the solution of DNA (or that

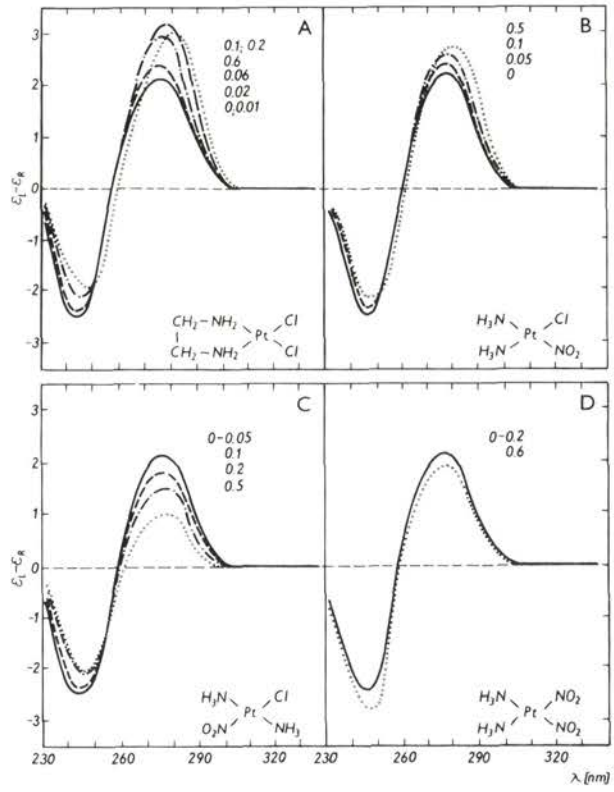


Fig. 1. The CD spectra of water - salt solutions of linear DNA in the presence of platinum compounds: (*A*) — [Pt(en)Cl_2]; (*B*) — *cis*-[$Pt(NH_3)_2NO_2Cl$]; (*C*) — *trans*-[$Pt(NH_3)_2NO_2Cl$]; (*D*) — *cis*-[$Pt(NH_3)_2(NO_2)_2$]. The values of r are given at the spectra.

of the DNA-Pt complex) with the PEG-containing solution (3 min; Chirana Labmixer, Czechoslovakia). The concentrations of PEG in the solutions of DNA microphase were 110 or 170 mg/ml.

Absorption spectra were recorded with a Specord M 40 spectrophotometer (GDR), CD spectra were recorded with a Jobin-Yvon Mark III dichrograph (France) using 1 cm cells; measurements were performed 24 h after the microphase was prepared.

Results

In the absorption spectrum of linear DNA molecules after the incubation with any of the platinum compounds studied a small increase in the absorption (by 7—14 % at the maximum of the band at about 260 nm) was observed when r was varied from 0.005 to 0.5. A bathochromic shift (2—4 nm) for all compounds (except *cis*-[$Pt(NH_3)_2(NO_2)_2$] and [$Pt(NH_3)_3Cl$]Cl) was also observed.

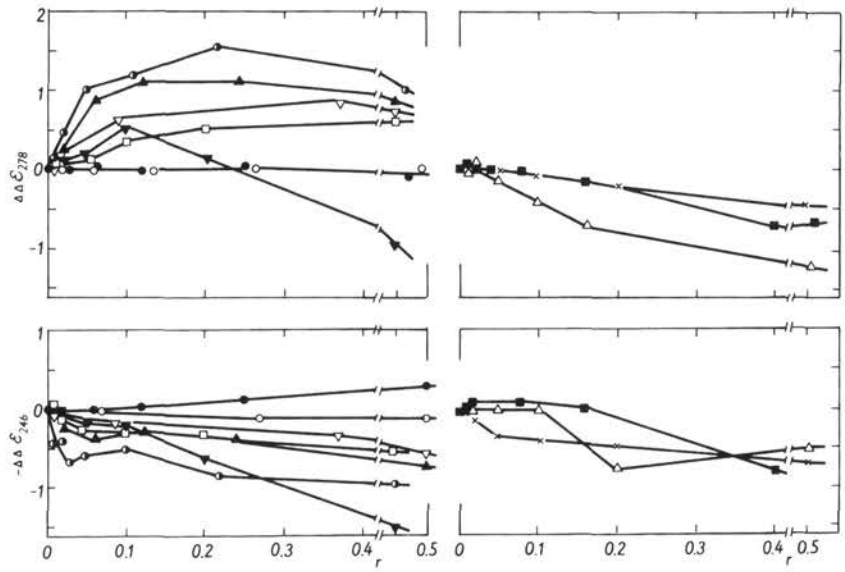


Fig. 2. The effect of platinum compounds on the amplitudes of the positive ($\lambda = 278$ nm) and the negative ($\lambda = 246$ nm) bands of the CD spectra of linear DNA molecules. Platinum compounds: ◑ — *cis*-$[Pt(NH_3)_2Cl_2]$; ▲ — $[Pt(en)Cl_2]$; △ — $[Pt(tetrameen)Cl_2]$; ▲ — *cis*-$[PtNH_3(Bz)Cl_2]$; ■ — *trans*-$[Pt(NH_3)_2Cl_2]$; □ — *cis*-$[Pt(NH_3)_2NO_2Cl]$; △ — *trans*-$[Pt(NH_3)_2NO_2Cl]$; ○ — *cis*-$[PtNH_3PyCl_2]$; ● — *cis*-$[Pt(NH_3)_2(NO_2)_2]$; x — $[Pt(NH_3)_3Cl]Cl$; $\Delta\Delta\varepsilon_{278}$ and $\Delta\Delta\varepsilon_{246}$ are equal to the difference of band amplitudes of a complex ($\Delta\varepsilon_r$) and free DNA ($\Delta\varepsilon_0$), $\Delta\varepsilon_r - \Delta\varepsilon_0$, at 278 and 246 nm, respectively.

Fig. 1 (*a*—*d*) illustrates the CD spectra of water - salt solutions of DNA after incubation with variable concentrations of platinum compounds $[Pt(en)Cl_2]$, *cis*- and *trans*-$[Pt(NH_3)_2NO_2Cl]$, and also *cis*-$[Pt(NH_3)_2(NO_2)_2]$. Fig. 2 shows the dependence of the amplitudes of the positive ($\lambda = 278$ nm) and negative ($\lambda =$ 246 nm) bands in the CD spectra of linear DNA on the r value for each platinum compound studied. The platinum compounds fit into several groups which are based on the character of changes in the CD spectrum of the DNA. For DNA complexes with *cis*-$[Pt(NH_3)_2Cl_2]$, $[Pt(en)Cl_2]$, $[Pt(tetrameen)Cl_2]$, *cis*-$[Pt(NH_3)_2NO_2Cl]$, and *cis*-$[PtNH_3(Bz)Cl_2]$ an increase in the amplitude of the positive band with the rise of r to 0.1 was observed; at further increases in r the amplitude of this band decreased. The greatest increase in the amplitude of the positive band was seen for *cis*-$[Pt(NH_3)_2Cl_2]$, the smallest for *cis*-$[PtNH_3(Bz)Cl_2]$. The amplitude of the negative band diminished with the growth of r. Note that the growth of r was accompanied by a slight bathochromic shift (3—5 nm) of the maxima of both bands.

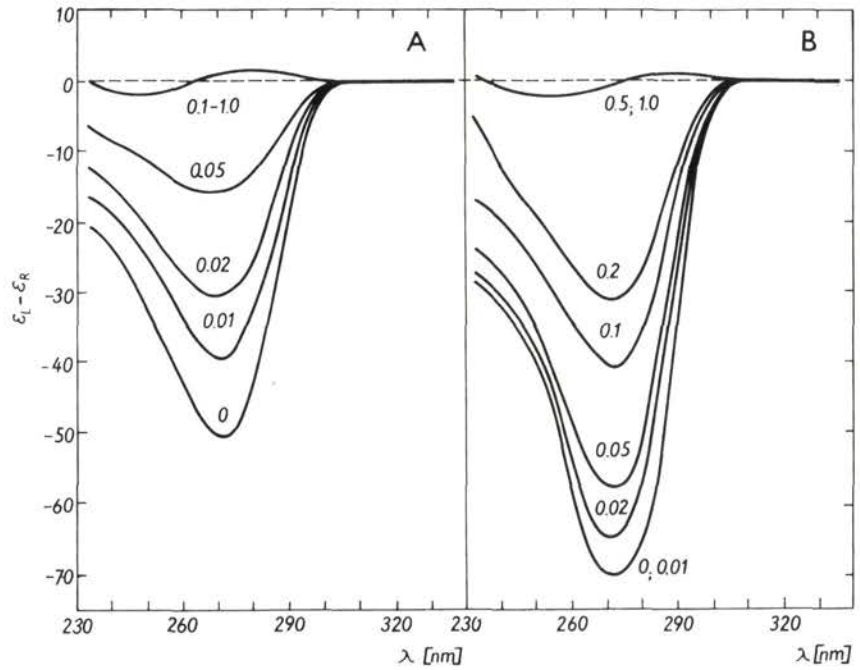


Fig. 3. The CD spectra of liquid crystalline microphases formed from free DNA and the complexes of DNA with *cis*-$[Pt(NH_3)_2NO_2Cl]$; (*A*) — 110 mg/ml PEG; (*B*) — 170 mg/ml PEG. The molecular mass of DNA was $\sim 0.8 \times 10^6$; the values of r are given at the spectra.

The compounds *trans*-$[Pt(NH_3)_2Cl_2]$, *trans*-$[Pt(NH_3)_2NO_2Cl]$, and $[Pt(NH_3)_3Cl]Cl$ induced a decrease of the amplitude of both bands ($\lambda = 278$ nm and $\lambda = 246$ nm) in the CD spectrum of linear DNA molecules at all r values; the value of this decrease varies, however, for different platinum compounds; the smallest changes were induced by $[Pt(NH_3)_3Cl]Cl$.

The CD spectra of linear DNA molecules were not significantly influenced by *cis*-$[Pt(NH_3)_2(NO_2)_2]$ and *cis*-$[PtNH_3PyCl_2]$.

The CD spectra of the liquid crystalline microphases formed from DNA molecules and complexes of DNA with *cis*-$[Pt(NH_3)_2NO_2Cl]$ at different r values are displayed in Fig. 3*a*, *b*. In the CD spectrum of the microphase from free DNA molecules ($r = 0$) at 110 mg/ml of PEG (Fig. 3*a*) an intense negative band was observed ($\lambda_{max} \approx 270$ nm). In the CD spectrum of the liquid crystalline microphase of the DNA - Pt complexes a decrease of the amplitude of this band occurred with an increase in r; at $r \geq 0.1$ the negative band vanished and the shape of the CD spectrum of the microphase was similar to that of linear non-condensed DNA molecules. In the CD spectrum of the microphase prepared from free DNA

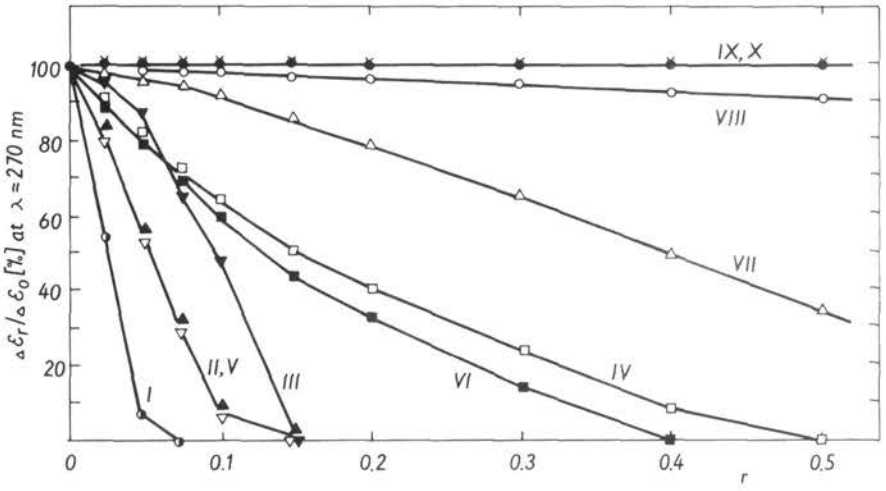


Fig. 4. The dependence of the relative amplitude of the negative band ($\Delta\varepsilon_r/\Delta\varepsilon_0$) in the CD spectra of microphases from the DNA (molecular mass $\sim 0.8 \times 10^6$) complexed with platinum compounds (170 mg/ml PEG) versus r. The symbols denoting platinum compounds are given in the legend to Fig. 2.

molecules and from the DNA complex with *cis*-$[Pt(NH_3)_2NO_2Cl]$ at a higher PEG concentration (170 mg/ml) (Fig. 3*b*), the amplitude of the negative band was larger than that in PEG at 110 mg/ml. With an increase in r this band was also diminished and a positive band appeared at $r \geq 0.5$.

The dependence of the relative intensity ($\Delta\varepsilon_r/\Delta\varepsilon_0$) of the negative band in the CD spectra of the microphases formed from complexes of DNA with platinum compounds on r is displayed in Fig. 4. It is evident from this figure that the amplitude of the negative band in the CD spectra of the microphases of the DNA complexed with *cis*-$[Pt(NH_3)_2Cl_2]$, $[Pt(en)Cl_2]$, [Pt(tetrameen)Cl_2], and *cis*-$[PtNH_3(Bz)Cl_2]$ diminished sharply with increasing r values and reached zero at $r < 0.2$. The dependence of the band amplitude on r for *trans*-$[Pt(NH_3)_2Cl_2]$, *cis*- and *trans*-$[Pt(NH_3)_2NO_2Cl]$ was less steep with $\Delta\varepsilon_r/\Delta\varepsilon_0$, assuming the zero value at $r \geqq 0.4$. The interaction of DNA with *cis*-$[PtNH_3PyCl_2]$ induced only a slight decrease of this band; a zero value was not observed even at the highest r values tested. The compounds $[Pt(NH_3)_3Cl]Cl$ and *cis*-$[Pt(NH_3)_2(NO_2)_2]$ did not substantially affect the intensity of the negative band in the CD spectrum of DNA microphase.

Table 2. Literature data on biological activity of platinum compounds

Compound	Biological activity	References
I *cis*-$[Pt(NH_3)_2Cl_2]$	Antimitotic and antitumour activities, cytogenetic effect. Phage inactivation.	(Rosenberg et al. 1969; Connors et al. 1972; Shooter et al. 1972; Ivanov et al. 1981b; Shevchenko et al. 1983)
II $[Pt(en)Cl_2]$	Antimitotic and antitumour activities. Phage inactivation.	(Rosenberg et al. 1969; Shooter et al. 1972; Ivanov et al. 1981b)
III *cis*-$[PtNH_3(Bz)Cl_2]$	Antimitotic activity.	(Ivanov et al. 1981a)
IV *cis*-$[Pt(NH_3)_2NO_2Cl]$	Antimitotic activity, cytogenetic effect.	(Ivanov et al. 1981b; Shevchenko et al. 1983)
V $[Pt(tetrameen)Cl_2]$	Selective antimitotic activity is not observed.	(Ivanov et al. 1976)
VI *trans*-$[Pt(NH_3)_2Cl_2]$	Selective antimitotic activity is absent. No antitumour activity.	(Rosenberg et al. 1969; Connors et al. 1972; Shooter et al. 1972; Ivanov et al. 1981b)
VII *trans*-$[Pt(NH_3)_2NO_2Cl]$	Selective antimitotic activity is absent. Cytogenetic effect is several times weaker than that of *cis*-isomer.	(Ivanov et al. 1981b; Shevchenko et al. 1977)
VIII *cis*-$[PtNH_3PyCl_2]$	Antimitotic activity and cytogenetic effect are absent.	(Ivanov et al. 1981a; Shevchenko et al. 1983)
IX *cis*-$[Pt(NH_3)_2(NO_2)_2]$	No antimitotic and antitumour activities. Cytogenetic effect is absent.	(Cleare and Hoeschele 1973; Ivanov et al. 1981b; Shevchenko et al. 1983)
X $[Pt(NH_3)_3Cl]Cl$	Antitumour activity is not observed.	(Cleare and Hoeschele 1973)

Discussion

It is of interest to compare the presented data concerning the effect of platinum compounds on the absorption and CD spectra of linear and condensed DNA molecules with the chemical structure and biological activity of platinum compounds.

The found changes in the absorption spectrum of DNA induced by interaction with the platinum compounds (not shown) correlate well with earlier observations (Horáček and Drobník 1971; Harder 1975; Macquet and Butour 1978a).

Table 2 summarizes data on the biological activity of the platinum compounds that were included in the present study. It is evident from Figs. 2 and 4 that the biologically active platinum compounds of the *cis*-dichlorodiamine type, *cis*-$[Pt(NH_3)_2Cl_2]$, $[Pt(en)Cl_2]$, and *cis*-$[PtNH_3(Bz)\,Cl_2]$, are similar in the character of their effect on the CD spectra of both linear DNA molecules and the liquid crystalline microphases of DNA. In the CD spectrum of linear DNA molecules

complexed with platinum compounds an increase in the amplitude of the positive band occurs followed by a decrease with increasing r values (see Fig. 2). Such changes in the CD spectrum have been shown to be characteristic of *cis*-bidentate covalent binding of platinum compounds to DNA nitrogen bases (Macquet and Butour 1978a, b), which is believed to be important for their antitumour activity. More recently evidence has been obtained that the increase in the amplitude of the positive CD band of DNA complexed with the biologically active platinum compounds reflects appearance of minor alterations, so-called premelting changes, in the secondary structure of DNA (Brabec et al. 1984; Kleinwächter and Rau 1984).

As to the CD spectrum of the liquid crystalline microphase of DNA, the picture is less complicated. In accordance with the theoretical viewpoint (Holzwarth and Holzwarth 1973; Grosberg 1980) the appearance of the intense band in the CD spectrum ($\lambda \approx 270$ nm) accompanies condensation of rigid linear DNA molecules and results from formation of helically twisted liquid crystalline microphase of DNA molecules. According to the theory (Holzwarth and Holzwarth 1973) and the experimental data (Evdokimow et al. 1975; Akimenko et al. 1977) the decrease of the intense negative band ($\lambda \approx 270$ nm) in the CD spectrum of the liquid crystalline microphase of DNA under influence of various agents (H^+, CH_3 group) reflects the disappearance of the helical twist of the liquid crystalline microphase only.

Thus, if liquid crystalline micophases are formed from DNA complexed with *cis*-$[Pt(NH_3)_2Cl_2]$, $[Pt(en)Cl_2]$, and *cis*-$[PtNH_3(Bz)Cl_2]$, a sharp drop of the amplitude of the negative band occurs in the CD spectra with an increase in r (Fig. 4).

cis-$[Pt(NH_3)_2NO_2Cl]$, in which one labile chloride group is replaced by a less labile NO_2 group, is also biologically active (Table 2). This compound affects the CD spectrum of the linear DNA molecules in a way similar to that of the platinum compounds discussed above, although its bidentate covalent binding is less probable. It must be noted, however, that *cis*-$[Pt(NH_3)_2NO_2Cl]$ differs markedly from the biologically active platinum compounds listed above in its effect on the CD spectrum of the DNA microphase: for the DNA - *cis*-$[Pt(NH_3)_2NO_2Cl]$ complex a smooth rather than a sharp decrease of the amplitude of the negative band was observed as r increased (Fig. 4).

By the character of changes in the CD spectrum of linear and condensed molecules of DNA (Figs. 2 and 4), $[Pt(tetrameen)Cl_2]$ resembles the antitumour active platinum compounds; however, this compound is unable to selectively inhibit mitosis (Table 2). This phenomenon remains unexplained.

For the other platinum compounds, antitumour activity and selective antimitotic effect were not observed (Table 2). The analysis of the results presented in Fig. 2 indicates that the biologically inactive platinum compounds either induce a decrease of the positive band amplitude only in the CD spectrum of linear DNA molecules, or fail to affect it at all. The effect of DNA complex formation with

these platinum compounds on the CD spectrum of the liquid crystalline microphase of DNA is either weak (*trans*-$[Pt(NH_3)_2Cl_2]$, *trans*-$[Pt(NH_3)_2NO_2Cl]$, and *cis*-$[PtNH_3PyCl_2]$), or absent (*cis*-$[Pt(NH_3)_2(NO_2)_2]$ and $[Pt(NH_3)_3Cl]Cl$) (Fig. 4).

The results reported here are in good agreement with the established observation that the presence of labile anionic ligands in the *cis* position is required for the specific biological activity of platinum compounds to become manifested. However, it has been found that if in *cis*-$[Pt(NH_3)_2Cl_2]$ a nonlabile NO_2 group is substituted for one chlorine atom, the platinum compound will not lose its biological activity (Ivanov et al. 1981b; Shevchenko et al. 1983). Only compounds with two *cis*-NO_2 groups appear to be biologically inactive.

It should be stressed, however, that the changes in CD spectra reported here are not related to the amount of platinum bound to DNA, but are plotted as a function of the total amount of platinum compounds present in the reaction mixture, expressed as r. Preliminary results (unpublished) show that all compounds investigated react with DNA and that the level of platinum binding is not influenced by the procedure used for the preparation of the liquid crystalline microphases. The ratio of bound to unbound platinum differs for different compounds, but the absence of any effect in the CD spectra, whether of linear DNA or its condensed form, cannot be attributed to the absence of interaction of the particular platinum compound with DNA.

Thus, the results obtained indicate that the specific biological action of platinum compounds of the *cis*-diamine type is predetermined by their polydentate binding to DNA. In addition to the *cis*-bidentate covalent binding, that has been referred to by several authors, a hydrogen bond formation may occur between DNA and both amino ligands by means of the protons at the nitrogen atoms. The fact that *cis*-$[Pt(NH_3)_2NO_2Cl]$ has antimitotic and mutagenic activities indicates that formation of one covalent and one or two hydrogen bonds is sufficient for the appearance of biological activity. The labilizing *trans*-effect (which is less for amino than for NO_2 groups) seems to play a role in the formation of hydrogen bonds. The differences in the *trans*-labilizing effectiveness might explain the absence of specific biological activity of *trans*-$[Pt(NH_3)_2NO_2Cl]$, *cis*-$[Pt(NH_3)_2(NO_2)_2]$, or $[Pt(NH_3)_3Cl]Cl$.

The fact that the CD spectra of liquid crystalline microphases are very sensitive to changes in the DNA secondary structure suggests that CD spectra may be used not only for the study of the mechanism of interaction of platinum compounds with DNA, but that they also may contribute to preliminary testing of potentially active compounds of this class.

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Received October 9, 1984/Accepted March 26, 1985