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DNA Interactions of a Novel Platinum Drug, *cis*-[PtCl(NH$_3$)$_2$(N7-Acyclovir)]$^+$

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ABSTRACT

We synthesized a novel platinum drug, *cis*-[PtCl(NH$_3$)$_2$(N7-ACV)]$^+$, in which ACV is the antiviral drug acyclovir [a deoxyriboguanosine analogue, 9-(2-hydroxyethoxymethyl)guanine]. This new compound exhibits antiviral efficacy *in vitro* and exhibits an antitumor activity profile different from that of cisplatin [*Metal-Based Drugs* **2:**249–256 (1995)]. To contribute to understanding the mechanisms underlying biological activity of this new compound, we studied modifications of natural and synthetic DNAs in cell-free media by *cis*-[PtCl(NH$_3$)$_2$(N7-ACV)]$^+$ by various biochemical and biophysical methods. The results indicated that the major DNA adduct of *cis*-[PtCl(NH$_3$)$_2$(N7-ACV)]$^+$ was a stable monofunctional adduct at guanine residues. In contrast to DNA adducts of other monodentate and clinically ineffective platinum(II) compounds, the adducts of *cis*-[PtCl(NH$_3$)$_2$(N7-ACV)]$^+$ terminated *in vitro* DNA and RNA synthesis. In addition, although DNA adducts of *cis*-[PtCl(NH$_3$)$_2$(N7-ACV)]$^+$ and cisplatin were different, some properties of DNA modified by either compound were qualitatively similar. Such similarities were not noticed if DNA modifications by other ineffective monofunctional platinum(II) complexes were investigated. Thus, the DNA binding mode of monofunctional *cis*-[PtCl(NH$_3$)$_2$(N7-ACV)]$^+$ was different from that of other monofunctional but ineffective platinum(II) complexes. It has been suggested that the unique capability of *cis*-[PtCl(NH$_3$)$_2$(N7-ACV)]$^+$ to modify DNA may be relevant to a distinct antitumor efficiency of this novel drug in comparison with cisplatin. It also has been suggested that at least some aspects of DNA interactions of *cis*-[PtCl(NH$_3$)$_2$(ACV)]$^+$ revealed in the current study could be exploited in the search for and development of new antiviral platinum complexes containing, as a part of the coordination sphere, antiviral nucleosides.

A number of platinum coordination compounds exhibit antiviral and antitumor activities (Taylor and Ward, 1991; Comis, 1994; Rixe *et al*., 1996). In the search for new, therapeutically more effective platinum drugs, platinum(II) compounds containing, as a part of the coordination sphere, certain selected antiviral nucleosides also were recently synthesized (Taylor and Ward, 1991; Coluccia *et al*., 1995). Several compounds of this type exhibit similar or enhanced antiviral activities *in vitro* and in many instances are less toxic to normal cells than either component (Taylor and Ward, 1991).

The synthesis of a novel compound, cisPt-ACV (Fig. 1C), was recently described (Coluccia *et al*., 1995). This compound is based on antitumor cisplatin [*cis*-diamminedichloroplatinum(II)] (Fig. 1A), which contains in its coordination sphere antiviral ACV [9-(2-hydroxyethoxymethyl)guanine, acycloguanosine] (Fig. 1C). The molecule of ACV contains an unchanged guanine residue and a modified, acyclic deoxyribose moiety. This antiviral drug exhibits high activity against various herpes viruses (O'Brien and Campoli-Richards, 1989).

Preliminary results indicated that cisPt-ACV retained anti-herpes simplex-1 efficiency *in vitro*, albeit less than that of the unplatinated ACV (Coluccia *et al*., 1995). This finding prompted us to assess systematically the *in vitro* antiviral activity of this new complex toward a variety of DNA and

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ABBREVIATIONS: ACV, acyclovir; cisPt-ACV, *cis*-[PtCl(NH$_3$)$_2$(*N*7-ACV)]$^+$ complex; dienPt, chlorodiethylenetriamineplatinum(II) chloride; DMS, dimethylsulfate; ELISA, enzyme-linked immunosorbent assay; FAAS, flameless atomic absorption spectrophotometry; RP-HPLC, reversed-phase high performance liquid chromatography; ICL, interstrand cross-link; r_i, molar ratio of free platinum complex to nucleotide phosphates at the onset of incubation with DNA; r_b, number of the molecules of platinum complex fixed per nucleotide residue; t_m, DNA melting temperature; transplatin, *trans*-diamminedichloroplatinum(II); transPt-ACV, *trans*-[PtCl(NH$_3$)$_2$(*N*7-ACV)]$^+$ complex.

RNA viruses. The results confirming activity of cisPt-ACV *in vitro* toward a number of viruses will be published in a separate communication.

Cisplatin (Fig. 1A) and its direct analogue carboplatin [*cis*-diammine(1,1-cyclobutyl-dicarboxylato)platinum(II)] are effective anticancer drugs currently approved for the treatment of several human carcinomas (Comis, 1994; Rixe *et al*., 1996). Even though these platinum drugs belong to the most successful antitumor compounds developed in recent years, they display limited activity against some of the common tumors, such as breast and colon carcinomas. In addition, a variety of adverse effects and acquired resistance are observed in patients receiving cisplatin or carboplatin chemotherapy. These limitations have inspired efforts to develop new platinum-based drugs that would display improved therapeutic properties.

Exploration of new structural classes of platinum antitumor drugs resulted in the discovery of various new platinum(II) complexes. These new compounds also include those of formula *cis*-$[PtCl(NH_3)_2(Am)]^+$ (where Am is an amine ligand). These formally monofunctional complexes (only containing one leaving chloride group), in which Am was a ligand derived from pyridine, pyrimidine, purine, piperidine, or aniline, demonstrated activity against a number of murine tumors and human tumor cell lines (Hollis *et al*., 1989, 1991) in contrast to closely related and simpler but inactive platinum/triamine complexes, such as dienPt [$[PtCl(H_2NCH_2CH_2NHCH_2CH_2NH_2)]Cl$] (Fig. 1B) or $[Pt(NH_3)_3Cl]Cl$. Therefore, the antitumor activity of cisPt-ACV also was tested (Coluccia *et al*., 1995). cisPt-ACV was found to be as effective as cisplatin when equitoxic doses were administered *in vivo* to P388 leukemia-bearing mice. Importantly, the cisPt-ACV also was active against a cisplatin-resistant subline of the P388 leukemia. This observation suggests that cisPt-ACV exhibits antitumor activity, but the mechanism underlying this activity is different from that of cisplatin.

We are currently testing the hypothesis that an alteration in DNA-binding mode may result in antitumor activity different from that of cisplatin. It is generally believed that the mechanism of anticancer activity of cisplatin and its simple analogues involves formation of platinum/DNA adducts that

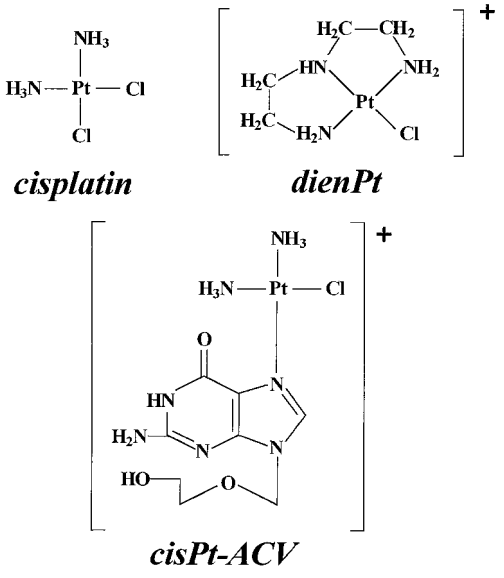

Fig. 1. Structures.

are capable of blocking DNA and RNA synthesis (for general reviews, see Johnson *et al*., 1989; Lepre and Lippard, 1990; Leng and Brabec, 1994) and induce programmed cell death (Barry *et al*., 1990; Ormerod *et al*., 1996). These platinum(II) complexes usually bind to DNA in a two-step process, producing first monofunctional adducts preferentially at the N(7) position of guanine residues, which can subsequently close to bifunctional lesions. There is a considerable evidence suggesting that the antitumor efficacy of cisplatin and its analogues is associated with the formation of DNA 1,2-intrastrand d(GpG) or d(ApG) cross-links by this drug (see, for example, Johnson *et al*., 1989; Lepre and Lippard, 1990; Leng and Brabec, 1994).

It is reasonable to expect that the molecular mechanism underlying antitumor activity of the compounds such as *cis*-$[PtCl(NH_3)_2(Am)]^+$ involves coordination of the platinum complex to DNA. On the other hand, no experimental data have been obtained indicating that such reaction is involved in the mechanism of antiviral activity of the platinated nucleoside analogues. Nevertheless, to address fundamental questions about the mechanism of biological action of platinum(II) compounds containing, as a part of the coordination sphere, certain selected antiviral nucleosides, studies were initiated on DNA interactions of cisPt-ACV in cell-free media. The results of these studies will contribute to understanding of the limits of the structure-activity relationships among platinum(II) complexes. It also is anticipated that at least some aspects of DNA interactions of *cis*-$[PtCl(NH_3)_2(Am)]^+$ revealed in this report will be useful when searching not only for new antitumor drugs but also for new antiviral platinum complexes containing, as a part of the coordination sphere, antiviral nucleosides.

Experimental Procedures

Materials. Cisplatin, transplatin, and dienPt were synthesized and characterized at Lachema (Brno, Czech Republic). cisPt-ACV and transPt-ACV, the *trans* isomer of cisPt-ACV] were prepared and characterized as described previously (Coluccia *et al*., 1995). The stock solutions of the platinum complexes were prepared through dissolution at a concentration of 5×10^{-4} M in 10 mM $NaClO_4$ in the dark at 25°; they were stored for ≥7 days before they were used. Calf thymus DNA (42% G + C, mean molecular mass ~ 2×10^7) also was prepared and characterized as described previously (Brabec and Paleèek, 1970). Plasmid pSP73 (2464 bp) was isolated according to standard procedures and banded twice in CsCl/ethidium bromide equilibrium density gradients. The oligodeoxyribonucleotides synthesized on an Applied Biosystem solid-phase synthesizer were purified by ion exchange fast performance liquid chromatography with a linear gradient of 0.1–0.8 M NaCl with 10 mM NaOH. In this report, the concentrations of oligonucleotides are related to the mononucleotide content. Restriction endonucleases were purchased from New England BioLabs (Beverly, MA). T4 polynucleotide kinase and Klenow fragment of DNA polymerase I were from Boehringer-Mannheim Biochemica (Mannheim, Germany). Riboprobe Gemini System II for transcription mapping containing T7 RNA polymerase was purchased from Promega (Madison, WI). Deoxyriboguanosine, DNase I from bovine pancreas, nuclease P1 from *Penicillium citrinum*, and alkaline phosphatase from calf intestine were from Sigma-Aldrich (Prague, Czech Republic). Ethidium bromide, acrylamide, bisacrylamide, urea, DMS, and NaCN were from Merck (Darmstadt, Germany). $TbCl_3 0{\cdot}6\ H_2O$ was from Fluka Chemie AG (Buchs, Switzerland). The radioactive products were from Amersham (Arlington Heights, IL).

Platination reactions. Calf thymus or plasmid DNAs were modified by platinum complexes in 10 mM $NaClO_4$ at 37° in the dark for 48 hr if not stated otherwise. In these samples, the number of platinum atoms fixed per nucleotide residue (r_b values) were determined by FAAS or differential pulse polarography (Kim *et al.*, 1990). The oligodeoxyribonucleotide duplex 5′-d(CTTCTCTTCTGGTCTTCTCT)/5′-d(GAGAGAAGACCAGAAGAGAA) [abbreviated as d(TGGT)/d(ACCA) according to its central sequences] was platinated in the following manner so it contained only a single adduct of cisPt-ACV. The single-stranded d(TGGT) at a concentration of 8×10^{-4} M [the top, pyrimidine-rich strand of the duplex d(TGGT)/d(ACCA)] was allowed to react with cisPt-ACV (the input molar ratio was 1 Pt atom per oligonucleotide strand) for 48 hr at 37°. The single product of this reaction was purified by ion exchange fast performance liquid chromatography (Brabec *et al.*, 1992) and further analyzed for the platinum content by FAAS. The concentration of the oligonucleotide present in the product, determined by absorption spectrophotometry, made it possible to conclude that the product of the reaction of single-stranded d(TGGT) contained 1 molecule of cisPt-ACV coordinated per oligonucleotide strand. The yield of this reaction was ~90%. The purified platinated or nonmodified d(TGGT) was further analyzed in the single-stranded form or annealed with the unplatinated bottom (complementary) strand, d(ACCA) (the mixture was incubated in 50 mM $NaClO_4$ at 65° for 5 min and subsequently slowly cooled at room temperature to ~22° within ~2 hr). The formation of the duplexes was checked by recording melting curves, which exhibited a clear cooperative helix-coil transition characterized by ~35% hyperchromic effect with a melting temperature of >50°.

Sequence specificity of DNA adducts. Transcription of the (*Nde*I/*Hpa*I) restriction fragment of pSP73 DNA with T7 RNA polymerase and electrophoretic analysis of transcripts was performed according to the protocols recommended by Promega (Promega Protocols and Applications, 43–46; 1989/90) and previously described in detail (Lemaire *et al.*, 1991; Brabec and Leng, 1993).

Fluorescence measurements. These measurements were performed with a Shimadzu RF 40 spectrofluorophotometer using a 1-cm quartz cell. Terbium fluorescence measurements were performed as follows: $TbCl_3$ was added to 8 μg of modified or control DNA/ml at a final concentration equivalent to twice the monomeric nucleotide content. The fluorescence intensity was measured after equilibration for 60 min at 25° in the dark. The excitation and emission wavelengths were 290 and 546 nm, respectively. Other details of these measurements can be found in earlier reports (Topal and Fresco, 1980; Balcarová and Brabec, 1989).

Unwinding of negatively supercoiled DNA. Unwinding of closed circular supercoiled pSP73 plasmid DNA was assayed by an agarose gel mobility shift assay (Keck and Lippard, 1992). The unwinding angle F, induced per platinum/DNA adduct, was calculated from the r_b value at which the complete transformation of the supercoiled to relaxed form of the plasmid was attained. Samples of pSP73 plasmid were incubated with cisPt-ACV at 37° in the dark for 48 hr. All samples were precipitated by ethanol and redissolved in the TBE (Tris-borate/EDTA) buffer. An aliquot of the precipitated sample was subjected to electrophoresis on 1% agarose gels running at 25° in the dark with TBE buffer with a voltage set at 30 V. The gels were then stained with ethidium bromide, followed by photography on Polaroid 667 film with transilluminator. The other aliquot was used for the determination of r_b values by FAAS.

ICL assay. cisPt-ACV at varying concentrations was incubated with 2 μg of pSP73 DNA linearized by *Eco*RI. The platinated samples were precipitated by ethanol and analyzed for DNA ICLs as described recently (Lemaire *et al.*, 1991; Brabec and Leng, 1993). The linear duplexes were first 3′-end labeled by means of the Klenow fragment of DNA polymerase I and [α-^{32}P]dATP. The samples were deproteinized by phenol and precipitated by ethanol, and the pellet was dissolved in 18 μl of a solution containing 30 mM NaOH, 1 mM EDTA, 6.6% sucrose, and 0.04% bromphenol blue. The amount of ICLs was analyzed by electrophoresis under denaturing conditions on alkaline agarose gel (1%). After the electrophoresis was completed, the intensities of the bands corresponding to single strands of DNA and ICL duplex were quantified by means of a Molecular Dynamics PhosphorImager (Storm 860 System with ImageQuant software; Sunnyvale, CA).

HPLC analyses. These analyses were performed using a Hitachi Series 4 liquid chromatograph equipped with a LCI-100 computing integrator and a Waters μBondapak C18 column. If not stated otherwise, the products were separated by RP-HPLC (isocratic elution with 0.1 M ammonium acetate, pH 5.5, in 3.9% CH_3CN at 1 ml/min flow rate). The following enzymatic digestion protocol was used to characterize the platinated deoxyribooligonucleotides. The samples (50 μg of the oligonucleotide) were incubated with 72 units of DNase I at 37°. After 4 hr, nuclease P1 (40 μg) was added, and the reaction was allowed to continue at 37° for 18 hr. Finally, alkaline phosphatase (39 units) was added, and the incubation continued for an additional 4 hr at 37°. The digested samples containing constituent nucleosides were heated for 2 min at 80° and centrifuged, and the supernatant was analyzed by RP-HPLC. The standard *cis*-[$Pt(NH_3)_2$(*N*7-ACV)(*N*7-dGuo)]$^+$ was prepared by reaction of deoxyriboguanosine (dGuo) with one equivalent of *cis*-[$PtCl(NH_3)_2$(*N*7-ACV)]NO_3. The latter species was generated by allowing the chloro form dissolved in distilled water to react with one equivalent of $AgNO_3$. The AgCl precipitate was removed by centrifugation. The resulting major product was purified by RP-HPLC using water/methanol gradient with 0.02 M ammonium acetate, pH 5.5, and its structure was confirmed by ^{1}H NMR spectroscopy and infrared spectroscopy. Proton NMR spectra were obtained with a Bruker AM 300 spectrometer and infrared spectra using Perkin-Elmer Cetus (Norwalk, CT) 283 and FT spectrophotometers.

Nessler colorimetric assay. Liberation of ammonia from cisPt-ACV on its binding to DNA was assayed using a classic Nessler reagent. After the reaction was completed, the absorbance of the samples was measured at 428 nm. The reaction was optimized so that ≥2% ammonia could be detected in the samples analyzed in this work. Other details of these analyses are given in the text below.

DMS footprinting. The chemical modifications of the platinated or nonmodified oligodeoxyribonucleotides, single-stranded d(TGGT) or double-stranded d(TGGT)/d(ACCA), by DMS were performed with the d(TGGT) strand 5′-end labeled by T4 polynucleotide kinase and [γ-^{32}P]ATP. Other details of these modifications were the same as in our previous work (Lemaire *et al.*, 1991; Brabec and Leng, 1993). Before analysis on a 24% polyacrylamide/8 M urea gel, the products were treated with 0.2 M NaCN (pH 11.5, 16 hr, 45° in the dark) to remove bound platinum. Intensities of the bands on the autoradiograms were quantified by means of a Molecular Dynamics PhosphorImager (Storm 860 System with ImageQuant software).

DNA melting. The melting curves of DNAs were recorded by measuring the absorbance at 260 nm. If not stated otherwise, the melting curves were recorded in media containing various concentrations of NaCl and 1 mM Tris·HCl with 0.1 mM EDTA, pH 7.4. The value of the melting temperature (t_m) was determined as the temperature corresponding to a maximum on the first-derivation profile of the melting curves. The t_m values could be thus determined with an accuracy of ±0.3°.

Immunochemical analysis. Monoclonal antibodies, Ab_{cis}, were prepared against double-helical calf thymus DNA modified by cisplatin at r_b of 0.08 in 10 mM $NaClO_4$ for 48 hr at 37°. They were purified and characterized in the same way as described previously (Sundquist *et al.*, 1987; Vrána *et al.*, 1992). Their specificity and avidity were the same as described previously (Sundquist *et al.*, 1987; Vrána *et al.*, 1992). The procedures for their immunoenzymatic analysis and ELISA also have been described (Sundquist *et al.*, 1987; Vrána *et al.*, 1992).

Other methods. UV spectra were measured with a Beckmann DU-8 spectrophotometer. FAAS measurements were carried out on a Unicam 939 AA spectrometer with a graphite furnace. For FAAS

analysis, DNA was precipitated with ethanol and dissolved in 0.1 M HNO_3.

Results and Discussion

Stability of cisPt-ACV and its DNA binding. We found conditions for RP-HPLC analysis that allowed quantitative detection of ACV in the presence of cisPt-ACV (Fig. 2A). The lower limit of this determination was 0.5% ACV in the presence of cisPt-ACV. cisPt-ACV was dissolved at concentrations of 1–5 $\times$ 10^{-4} M in water, 0.01 M and 1.0 M $NaClO_4$ or NaCl and incubated in the dark at 37° for 1 week. No peak in the RP-HPLC profile corresponding to free ACV was observed, indicating that no ACV spontaneously dissociated from cisPt-ACV in any of the solutions. Solutions of calf thymus DNA at a concentration of 0.32 mg/ml were incubated with cisPt-ACV at r_i values of 0.01–0.1 in 10 mM $NaClO_4$ at 37° (r_i is defined as the molar ratio of free platinum complex to DNA nucleotide phosphates at the onset of incubation with DNA). At various time intervals, an aliquot of the reaction mixture was withdrawn and precipitated by ethanol, and the supernatant was assayed by RP-HPLC for free cisPt-ACV (not bound to DNA). In this way, we also were able to check directly whether cisPt-ACV on its binding to double-helical DNA is decomposed so its ACV moiety is released into the solution. The HPLC peak corresponding to the free (unbound) cisPt-ACV complex decreased with time of its incubation with DNA and completely disappeared after 24 hr [half-time of this reaction ($t_{1/2}$) was~1.7 hr] (shown for r_i = 0.1 in Fig. 2B). This result indicates that cisPt-ACV binds to DNA with a similar rate as cisplatin (Kim *et al*., 1990) and that all molecules of cisPt-ACV are bound to DNA within 24 hr. No peak appeared in the HPLC profile corresponding to the released ACV after 24 hr of the incubation. It was verified that a small quantity of free ACV (corresponding to 1% of the total amount of cisPt-ACV present in the reaction with DNA) added to this DNA sample after incubation lasting 24 hr yielded a well defined peak in the HPLC profile appearing at the same retention time as free ACV (Fig. 2A). The samples of DNA, to which all molecules of Pt-ACV present in the reaction mixture were bound after incubation in 10 mM $NaClO_4$ for 24 hr, were further incubated for additional 48 hr at 37° in 0.01, 0.05, and 1 M $NaClO_4$ or in 0.05 and 1 M NaCl. These subsequent incubations resulted in only the occurrence of a very small peak coeluting with ACV, indicating that only a negligible amount of ACV was released; the amount of ACV released did not exceed ~3%.

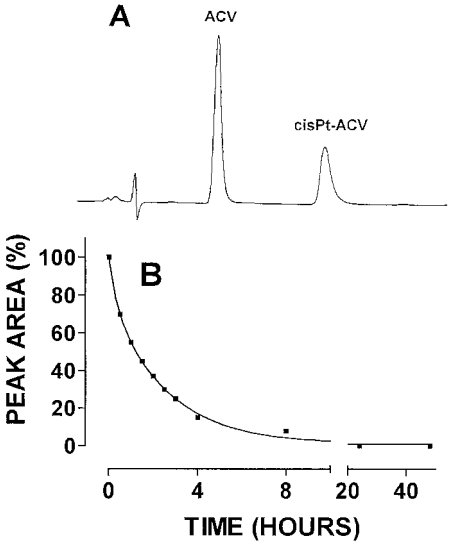

Fig. 2. A, C18 RP-HPLC separation of ACV and cisPt-ACV. B, Dependence of the area under the HPLC peak of cisPt-ACV (see A) mixed with 32 μg of calf thymus DNA/ml at r_i = 0.1 on the time (for other details, see the text). The peak area at the onset of incubation of cisPt-ACV with DNA was taken as 100%.

In addition, we investigated labilization of ammonia in free cisPt-ACV or in adducts formed by cisPt-ACV on double-helical DNA by means of an assay using Nessler reagent. No ammonia was found in the solutions of 5 $\times$ 10^{-4} M cisPt-ACV dissolved in water or 0.01, 0.05, and 1 M $NaClO_4$ or in 0.05 and 1.0 M NaCl. In addition, no ammonia was liberated in the presence of excess of double-helical DNA (0.032 mg/ml, r_i = 0.1 in 10 mM $NaClO_4$ at 37°) within 24 hr or even after prolonged incubations (additional 5 days at 37° in 0.01–1 M $NaClO_4$ or NaCl); it was verified that the assay allowed determining amounts of ammonia as small as 2% under the conditions used. The same binding rate was noticed if DNA was incubated with transPt-ACV and free platinum assayed by differential pulse polarography (Kim *et al*., 1990).

Solutions of calf thymus DNA were incubated under identical conditions with cisPt-ACV or transPt-ACV using fresh stock solutions (added to DNA samples immediately after dissolving the platinum complex). No changes in DNA binding were observed compared with the experiments in which the same stock solutions stored ≥1week were used. In addition, pH of the reaction mixture containing DNA and cisPt-ACV or transPt-ACV was measured within 24 hr after mixing DNA with the platinum complex, and no changes in pH were noticed.

Sequence specificity of cisPt-ACV binding to DNA. Transcription mapping studies were performed to determine the preferential binding of cisPt-ACV to specific sites or regions in DNA. Recent work has shown that the *in vitro* RNA synthesis by RNA polymerases on DNA templates containing several types of bidentate adducts of platinum complexes can be prematurely terminated at the level or in the proximity of adducts (Lemaire *et al*., 1991; Brabec and Leng, 1993; Brabec *et al*., 1994; Nováková *et al*., 1995). Importantly, monofunctional DNA adducts of several platinum(II) complexes are unable to terminate RNA synthesis (Lemaire *et al*., 1991; Brabec and Leng, 1993; Brabec *et al*., 1994).

Cutting of pSP73 DNA by *Nde*I and *Hpa*I restriction endonucleases yielded a 221-bp fragment containing T7 RNA polymerase promotor in the upper strand close to the 3′-end of the fragment (Lemaire *et al*., 1991; Brabec and Leng, 1993). The experiments were carried out using this linear DNA fragment, modified by cisplatin, dienPt, or cisPt-ACV at r_b = 0.01, for RNA synthesis by T7 RNA polymerase (Fig. 3A) (r_b is defined as the number of platinum atoms fixed per nucleotide residue). RNA synthesis on the template modified by cisplatin yielded fragments of defined sizes, which indicates that RNA synthesis on DNA modified by these platinum complexes was prematurely terminated. The major stop sites observed for cisplatin (Fig. 3A, *cisDDP*) were at d(GG) or d(AG) sites. In contrast, RNA synthesis on the template modified by monofunctional dienPt yielded no fragments that would correspond to premature termination of the RNA synthesis (Fig. 3A, *dienPt*). The results obtained with the frag-

ments modified by cisplatin or dienPt were in agreement with previous reports (Lemaire *et al.*, 1991; Brabec and Leng, 1993; Brabec *et al.*, 1994). Importantly, RNA synthesis on the template globally modified by cisPt-ACV (Fig. 3A, *cisPt-ACV*) was clearly terminated although somewhat less efficiently than the synthesis on the template modified by cisplatin. The major stop sites observed for cisPt-ACV were distributed less regularly [i.e., not only at single dG sites in some d(GG) or 5′-d(AG)-3′ sequences, which are preferential DNA binding sites of cisplatin (Fichtinger-Schepman *et al.*, 1985; Eastman, 1987), but also at single dG sites in various other sequences] (Fig. 3B). Importantly, RNA synthesis on the unplatinated template in the presence of ACV at r_i = 0.01 and 0.05 yielded no termination sites (not shown).

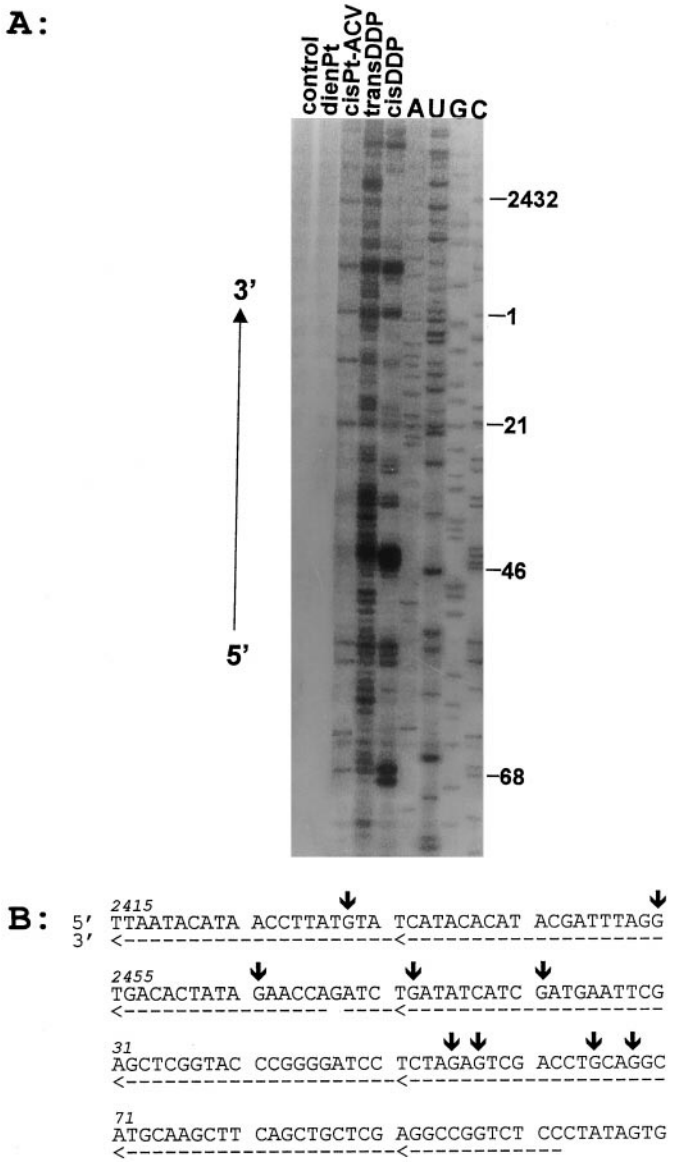

Fig. 3. A, Autoradiogram of 6% polyacrylamide/8 M urea sequencing gel showing inhibition of RNA synthesis by T7 RNA polymerase on the *Nde*I/*Hpa*I fragment of pSP73 plasmid modified by platinum complexes. *Control*, nonmodified template. *dienPt*, the template modified by dienPt at r_b = 0.05. *cisPt-ACV*, the template modified by cisPt-ACV at r_b = 0.01. *transDDP*, the template modified by transplatin at r_b = 0.01. *cisDDP*, the template modified by cisplatin at r_b = 0.01. *U*, *A*, *C*, and *G*, chain terminated marker RNAs. *Numbers*, nucleotide sequence numbering of B. B, Portion of the base sequence used to monitor inhibition of RNA synthesis on the template containing the adducts of Pt-ACV and other platinum complexes. Only the upper strand of the template is given. *Dashed line*, sequence transcribed by T7 RNA polymerase, which uses the upper strand of the *Nde*I/*Hpa*I fragment of pSP73 plasmid as a template. *Arrows*, Stop signals for DNA modified by cisPt-ACV. *Numbers*, nucleotide numbering in the sequence map of pSP73 plasmid.

The most striking feature of these transcription experiments is that the adducts in DNA globally modified by cisPt-ACV prematurely terminate RNA synthesis. This is in remarkable contrast with RNA synthesis on the template modified by monofunctional platinum complexes, such as dienPt or $[Pt(NH_3)_3Cl]Cl$ or monofunctional adducts of cisplatin or transplatin (Lemaire *et al.*, 1991; Brabec and Leng, 1993; Brabec *et al.*, 1994). In addition, it is generally accepted that monofunctional DNA adducts of cisplatin are not relevant to the cytostatic effects of this metal complex and that monofunctional dienPt or $[Pt(NH_3)_3Cl]Cl$ exhibits no anticancer activity. Thus, the results of the transcription mapping experiments (Fig. 3) are consistent with the hypothesis that cisPt-ACV forms some type of monofunctional DNA adduct that modifies DNA in a manner fundamentally different from that of monofunctional dienPt or $[Pt(NH_3)_3Cl]Cl$.

Transcription mapping has revealed (Fig. 3) that d(GG) sequence is among those at which cisPt-ACV is bound. The d(GG) site in double-helical DNA is a preferential binding site of antitumor cisplatin, at which this bifunctional drug forms the 1,2-intrastrand cross-link. To further corroborate the conclusion that the DNA binding mode of cisPt-ACV is different from that of cisplatin, chemical studies of a synthetic oligodeoxyribonucleotide duplex containing a single adduct of cisPt-ACV at the d(GG) site were conducted.

The duplex d(TGGT)/d(ACCA) (19 base pairs) was designed (see Experimental Procedures); it contains in the top pyrimidine-rich strand a central d(GG) sequence. The single-stranded d(TGGT) [the top, pyrimidine-rich strand of the duplex d(TGGT)/d(ACCA)] was modified so it contained a single adduct of cisPt-ACV (see Experimental Procedures). The platinated sites in this oligonucleotide then were identified using enzymatic digestion analysis. The platinated or nonmodified oligonucleotides d(TGGT) were treated with DNase I, P1 nucleases, and finally with alkaline phosphatase to yield corresponding deoxyribonucleosides. An RP-HPLC analysis of the products of this enzymatic digestion procedure is shown in Fig. 4. Peaks C, G, and T are nonmodified deoxyribocytidine, deoxyriboguanosine, and thymidine, respectively, assigned by coinjection with samples of pure deoxyribonucleosides. The *cis*-$[Pt(NH_3)_2(N7\text{-ACV})(dG)]^{2+}$ cation, prepared by reaction of *cis*-$[Pt(NH_3)_2(N7\text{-ACV})(Cl)]Cl$ with dG, was used to assign peak X by coinjection. Because this is the only new product observed in the enzymatic digestion analysis, it can be concluded that product X is a monofunctional adduct of cisPt-ACV at a guanine residue. This conclusion is further supported by the observation (Fig. 4) that the area under the peak G yielded by the platinated oligonucleotide was exactly half of that found for the nonmodified, control sample. Nevertheless, the HPLC enzymatic digestion analysis could not determine which of the two guanine residues in the TGGT sequence was a preferential binding site for the monofunctional coordination of cisPt-ACV to DNA.

Further insight into the nature of the single cisPt-ACV adduct in single-stranded oligonucleotide d(TGGT) was provided by DMS footprinting of platinum coordinated to DNA.

This assay is based on the fact that DMS methylates the N7 position of guanine residues in DNA, producing alkali-labile sites (Lemaire *et al*., 1991; Brabec and Leng, 1993). However, if N7 is covalently bound to platinum, it cannot be methylated. The platinated and nonmodified d(TGGT) strands were 5′-end labeled with ^{32}P and subsequently reacted with DMS. The oligonucleotides then were treated with NaCN and hot piperidine and analyzed by denaturing polyacrylamide gel electrophoresis (Fig. 5, *ss noPt and ss Pt*), and the autoradiograms were evaluated by densitometry. For the nonmodified oligonucleotide, shortened fragments due to the cleavage of the strand at the two methylated guanine residues were observed in the gel (Fig. 5, *ss noPt*). For the oligonucleotide containing the single adduct of cisPt-ACV, shortened fragments due to the cleavage of the strand at the two methylated guanine residues also were observed, but their intensity was exactly half of those of the nonmodified oligonucleotide (Fig. 5, *ss Pt*). The same result was obtained if the platinated oligomer was incubated before reaction with DMS for 48 hr in 0.05 and 1.0 M $NaClO_4$ or NaCl. The only reasonable explanation of this result is that cisPt-ACV forms stable monofunctional adducts without any preference at either guanine residue in the central sequence TGGT of the oligomer 20 nucleotides long.

The same studies based on enzymatic digestion analysis and DMS footprinting were performed with the oligomer d(TGGT) (containing the single adduct of cisPt-ACV and characterized as described in the preceding paragraphs), which was further hybridized with its complementary strand d(ACCA). The goal of these studies was to show whether the single monofunctional adducts formed by cisPt-ACV at either guanine residues of the single-stranded d(TGGT) oligonucleotide also are stable in double-helical DNA. The bottom complementary strand was added to either the nonmodified or platinated d(TGGT), and the mixture of the two oligonucleotides in the medium of 50 mM $NaClO_4$ was incubated to form the duplex (see Experimental Procedures). Both platinated and nonmodified oligonucleotide duplexes d(TGGT)/d(ACCA) were treated with the enzymes to yield corresponding deoxyribonucleosides in the same way as single-stranded d(TGGT). The peaks C, G, T, and A in the HPLC profiles (Fig. 4B) are nonmodified deoxyribocytidine, deoxyriboguanosine, thymidine, and deoxyriboadenosine, respectively, assigned by coinjection with samples of pure deoxyribonucleosides. The profile obtained for the platinated duplex only contained one new peak X, identified as *cis*-$[Pt(NH_3)_2(N7\text{-ACV})(dG)]^{2+}$ by coinjection with authentic material. Thus, these results suggest that the monofunctional adduct of cisPt-ACV is not converted to a bifunctional lesion in double-helical DNA. This conclusion is further supported by the observation (Fig. 4B) that the area under the peak G yielded by the platinated duplex was exactly eight-ninths of that found for the nonmodified, control duplex (containing nine guanine residues).

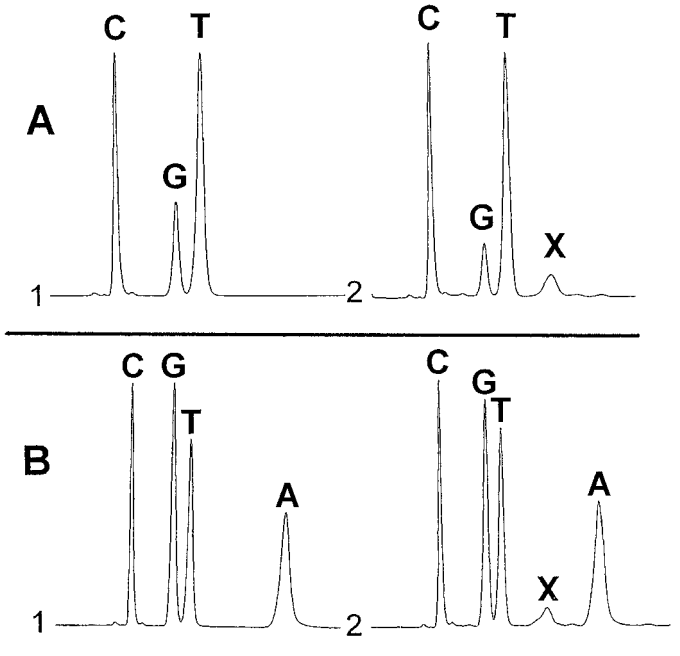

Fig. 4. RP-HPLC separation of the products of enzymatic digest of (A) single-stranded oligonucleotide d(TGGT) nonmodified (*curve 1*) or containing a single adduct of cisPt-ACV (*curve 2*). B, Oligonucleotide duplex d(TGGT)/d(ACCA) nonmodified (*curve 1*) or containing a single adduct of cisPt-ACV in the top strand, d(TGGT) (*curve 2*). For other details, see the text.

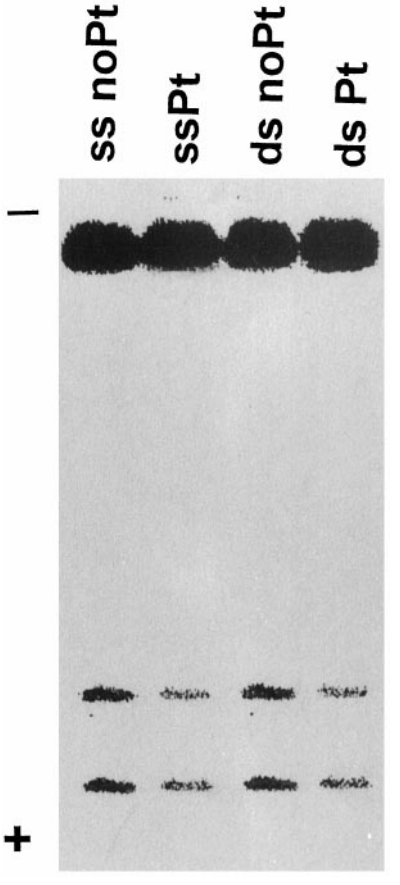

Fig. 5. A, Autoradiogram of 24% polyacrylamide/8 M urea gel showing piperidine-induced specific strand cleavage at DMS-modified bases in unplatinated single-stranded oligonucleotide d(TGGT) (*ss noPt*), single-stranded d(TGGT) containing the single adduct of cisPt-ACV (*ss Pt*), unplatinated oligonucleotide duplex d(TGGT)/d(ACCA) (*ds noPt*), and the duplex d(TGGT)/d(ACCA) containing the single adduct of cisPt-ACV in the top strand d(TGGT). The analysis of the single-stranded d(TGGT) was performed with the strand radioactively labeled at its 5′ end. The analysis of the duplexes d(TGGT)/d(ACCA) was performed with their top strands 5′-end labeled, so *ds noPt* and *ds Pt* in this figure are relative to the top strand. *ss Pt and ds Pt*, relative to the oligomers containing the single adduct of cisPt-ACV, which was, after the modification by DMS, subsequently treated with 0.2 M NaCN (alkaline pH) for 10 hr at 45° to remove platinum from the duplex. See the text for other details.

In further experiments, the platinated and nonmodified d(TGGT) single strands 5′-end labeled with ^{32}P were hybridized with the complementary strand d(ACCA) (which was not radioactively labeled). These duplexes were reacted with DMS and further analyzed by gel electrophoresis (Fig. 5, *ds noPt and ds Pt*) in the same way as single-stranded oligonucleotides (*vide supra*). The intensities of the bands yielded by the two guanine residues contained in the top strand of the platinated duplex were half of those of the nonmodified duplex. It was verified (not shown) that the intensities of these bands were lowered in the same extent if the duplexes were reacted with a lower amount of DMS, which methylated only 10% molecules of the unplatinated duplex (in contrast to 20% in the experiment demonstrated in Fig. 5). The same result was obtained when the platinated duplex was incubated before reaction with DMS for 48 hr in 1.0 M $NaClO_4$ or 0.05 and 1.0 M NaCl. This result is consistent with the view that the monofunctional adducts of cisPt-ACV formed at guanine residues are stable in double-helical DNA. Taken together, the results of the current study clearly demonstrate that monofunctional coordination of cisPt-ACV to single-stranded or double-helical DNA results in no elimination of ACV or NH_3 ligands, which could allow closure of these monofunctional adducts to bifunctional lesions.

Unwinding induced in DNA by cisPt-ACV binding. Electrophoresis in native agarose gel was used to determine the unwinding induced in pSP73 plasmid by cisPt-ACV through monitoring of the degree of supercoiling (Keck and Lippard, 1992) (Fig. 6). A compound that unwinds the DNA duplex reduces the number of supercoils. This decrease in supercoiling (on binding of unwinding agents) causes a decrease in the rate of migration through agarose gel, which makes it possible for the unwinding to be observed and quantified. Fig. 6 shows electrophoresis gels in which increasing amounts of cisPt-ACV have been bound to a mixture of relaxed and supercoiled pSP73 DNA. The unwinding angle is given by $F = 18\ s/r_b(c)$, where s is the superhelical density and $r_b(c)$ is the value of r_b at which the supercoiled and relaxed forms comigrate (Keck and Lippard, 1992). Under the current experimental conditions, s was calculated to be −0.063 on the basis of the data of cisplatin for which the $r_b(c)$ was determined in this study and F was taken 13° (Keck and Lippard, 1992). Unwinding angles for cisPt-ACV calculated in this way were 6°. The unwinding angles for cisplatin and dienPt taken from the literature (Bellon *et al.*, 1991; Keck and Lippard, 1992) are 13° and 6°, respectively.

Although the molecular details of the interactions of cisPt-ACV with DNA are not fully understood, the information obtained from the unwinding experiments is nevertheless valuable. The values of unwinding angles are affected by the nature of the ligands in the coordination sphere of platinum and the stereochemistry at the platinum center. A previous systematic work by others (Keck and Lippard, 1992) revealed that the platinum(II) compounds fall into different classes according to their DNA binding modes. It has been shown that platinum(II) compounds with the smallest unwinding angles (6°) are those that can bind DNA only monofunctionally [dienPt or $[Pt(NH_3)_3Cl]Cl$]. The observation that cisPt-ACV, which unwound DNA by 6°, can be grouped with other monofunctional platinum(II) compounds is readily understood in terms of an adduct structure in which cisPt-ACV is preferentially coordinated to DNA in a monodentate manner. In this configuration, the ACV ligand in cisPt-ACV either has only little or no opportunity to interact with the double helix or interacts in such a way that it does not contribute to the unwinding induced by this complex.

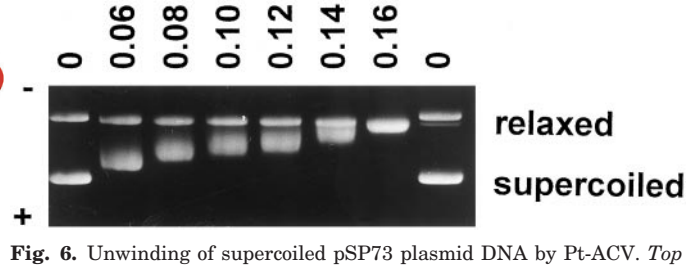

Fig. 6. Unwinding of supercoiled pSP73 plasmid DNA by Pt-ACV. *Top bands*, form of relaxed plasmid. *Bottom bands*, closed, negatively supercoiled plasmid. The plasmid was incubated with Pt-ACV for 48 hr at r_b values indicated (*upper lanes*).

DNA ICL by cisPt-ACV. On electrophoresis in agarose gel under denaturing conditions, 3′-end labeled strands of linearized pSP73 plasmid containing no ICLs migrate as a 2464-base single strand, whereas the ICL strands migrate more slowly as a species of higher molecular mass (Lemaire *et al.*, 1991; Brabec and Leng, 1993). The weak bands corresponding to more slowly migrating ICL fragments were only noticed if cisPt-ACV complex was used to modify DNA in linearized form at r_b of 0.002 (not shown). The intensity of the more slowly migrating band increased with the growing level of the modification. The radioactivity associated with the individual bands at each r_b was measured to obtain estimates of the fraction of ICL molecules. These estimates gave the frequency of ICLs formed by cisPt-ACV in linear DNA (amount of ICLs/molecule of cisPt-ACV complex bound to DNA) of only ~0.2% almost independently of r_b. This result indicates that the ICL efficiency of cisPt-ACV in linear DNA is negligible [cisplatin forms under identical conditions ~6% ICLs (Brabec and Leng, 1993; Vrána *et al.*, 1995)].

Melting of DNA modified by cisPt-ACV. Calf thymus DNA was modified by cisPt-ACV, cisplatin, or dienPt to various r_b (0–0.2) (at 37° for 48 hr so all molecules of the platinum complexes were coordinated to DNA). The effect of these modifications on DNA melting temperature, t_m, was measured (Fig. 7). The results indicate that cisPt-ACV has a similar effect on DNA melting as cisplatin (i.e., both complexes reduce thermal stability of DNA). In contrast, clinically ineffective monofunctional dienPt or bidentate transplatin enhances thermal stability of DNA under identical conditions. The observation that the t_m values of DNA modified by cisPt-ACV were decreased is consistent with an occurrence of conformational alterations induced in DNA by cisPt-ACV that destabilize the duplex (Žaludová *et al.*, 1996).

Fluorescence of complexes of terbium ion with DNA modified by cisPt-ACV. Terbium ion (Tb^{3+}) fluorescence is used to investigate local perturbations induced in conformation of double-helical DNA by various physical or chemical agents, including cisplatin pimprivate ENRf8 (Arquilla *et al.*, 1983; Balcarová and Brabec, 1989). This assay is based on the observation that Tb^{3+} fluorescence is strongly enhanced when the ion is bound to the N7 atoms of guanine residues in distorted DNA regions pimprivate ENRf8 (Topal and Fresco, 1980). The modification of double-helical DNA by cisplatin has been shown to result in substantial increase of the fluorescence of this lanthanide cation (Arquilla *et al.*, 1983; Bal-

carová and Brabec, 1989). This enhancement takes place due to its binding to unplatinated guanine residues in distorted regions around the platination site (Balcarová and Brabec, 1989). In contrast, the coordination of ineffective complexes, such as transplatin or monofunctional dienPt to double-helical DNA results in no distortions, which would increase the accessibility of base residues for their reaction with terbium leading to enhancement of its fluorescence (Arquilla *et al*., 1983; Balcarová and Brabec, 1989).

Calf thymus DNA was modified by cisplatin, dienPt, and cisPt-ACV at r_b values in the range of 0–0.1, the resulting samples were treated with $TbCl_3$, and the fluorescence was measured as described in Experimental Procedures (Fig. 8). In accordance with previous results (Arquilla *et al*., 1983; Balcarová and Brabec, 1989), the modification by cisplatin resulted in a marked enhancement of terbium fluorescence, whereas the modification by dienPt had only a negligible effect. The modification by cisPt-ACV also resulted in a significant enhancement of the fluorescence, but the fluorescence intensity was considerably lower than that seen in the case of DNA modified by cisplatin. These results can be interpreted to mean that cisPt-ACV induces in double-helical DNA local conformational alterations that are, at least in some features, similar to those induced by cisplatin. The extent of the conformational changes induced by cisPt-ACV (as detected by terbium fluorescence) is, however, considerably lower than that induced by cisplatin. On the other hand, this observation is in contrast to the modification of DNA by monofunctional dienPt, which does not induce in DNA this type of distortions.

Immunochemical analysis of DNA modified by cisPt-ACV. The antibodies raised against DNA modified by cisplatin have been found useful for probing the structures of DNA adducts formed by a number of different platinum complexes (e.g., Sundquist *et al*., 1987; Hollis *et al*., 1991; Vrána *et al*., 1992; Nováková *et al*., 1995; Vrána *et al*., 1995; Žaludová *et al*., 1997). We prepared antibodies that bind specifically to DNA modified by cisplatin and its analogues (Ab_{cis}). Ab_{cis} were found to exhibit strict requirements for two neighboring purine residues in one strand of DNA coordinated in *cis* geometry with the platinum atom of *cis*-$[Pt(NH_3)_2]^{2+}$ unit; moreover, this antibody is insensitive to the chemical nature of the inert *cis* platinum amine ligand. Importantly, Ab_{cis} do not recognize DNA adducts of transplatin and monofunctional adducts of several platinum(II) complexes (Sundquist *et al*., 1987; Vrána *et al*., 1992).

Several results indicating that cisPt-ACV does not form bifunctional adducts on DNA through loss of an amine ligand have been presented (*vide supra*). We intended to support this conclusion by using Ab_{cis}. Using competitive ELISA, the inhibition of the binding of Ab_{cis} to their immunogens (calf thymus DNA modified by cisplatin at r_b = 0.08 for 48 hr, respectively) by DNA modified by cisPt-ACV to various r_b values in the range of 0.005–0.1 was measured (Fig. 9). The results (Fig. 9) demonstrate that Ab_{cis} recognized DNA modified by cisPt-ACV in a broad range of r_b values (0.01–0.1) (shown in Fig. 9 for r_b = 0.05) although somewhat less efficiently than DNA modified by cisplatin.

To explain this observation, we have taken into consideration the fact that cisPt-ACV preferentially forms on double-helical DNA stable monofunctional adducts at guanine residues. If this type of DNA binding occurs, then the platinum atom in cisPt-ACV still coordinates two guanine residues in *cis* position: the guanine residue contained in the ACV and the guanine residue in DNA to which cisPt-ACV is monodentately coordinated. Apparently, the structural motif recognized by Ab_{cis} could also occur in double-helical DNA due to the monofunctional binding of the cisPt-ACV. Consistent with this view is the observation that Ab_{cis} do not recognize DNA modified by *cis*-$[PtCl(NH_3)_2(Am)]^+$, where Am is an amine ligand derived from pyrimidine derivatives (Hollis *et al*., 1991). Thus, it seems reasonable to suggest that DNA adducts of cisPt-ACV mimic to some extent the structural motif produced by the major DNA adduct of cisplatin.

Conclusions. A new platinum(II) antiviral and antitumor agent, *cis*-$[Pt(NH_3)_2(Am)Cl]^+$, was synthesized, in which Am is the known antiviral agent ACV (Coluccia *et al*., 1995). The current results suggest that this new compound can coordinate to DNA and preferentially forms in double-helical DNA stable monofunctional adducts at guanine residues. The re-

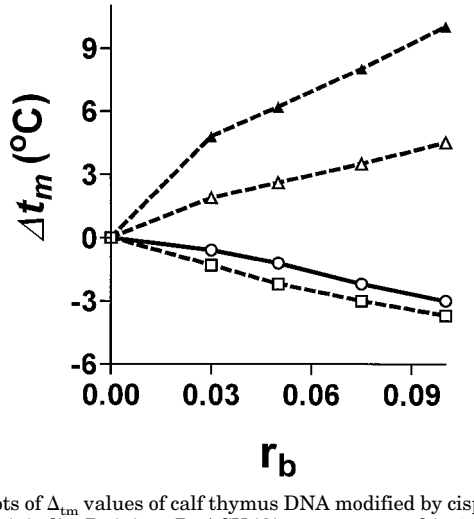

Fig. 7. Plots of Δ_{tm} values of calf thymus DNA modified by cisplatin (□), transplatin (▲), dienPt (△), or Pt-ACV (○) on r_b measured in 10 mM NaCl plus 1 mM Tris·HCl with 0.1 mM EDTA, pH 7.4 (Δt_m is defined as the difference between the t_m values of nonmodified and platinated DNAs).

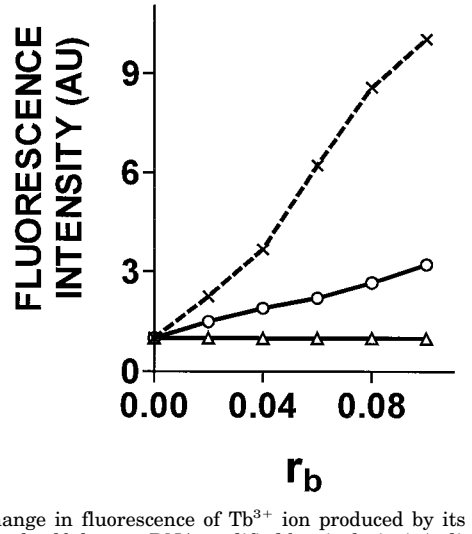

Fig. 8. Change in fluorescence of Tb^{3+} ion produced by its binding to double-helical calf thymus DNA modified by cisplatin (×), dienPt (△), or cisPt-ACV (○) at various r_b or by its mixing with DNA to which ACV was added at various r_i values. Fluorescence of untreated DNA was arbitrarily set at unity.

sults also demonstrate that cisPt-ACV, when monodentately coordinated at d(GG) sites in single-stranded or double-helical DNA, is bound with equal preference to either guanine residue. This is in contrast to DNA binding mode of the similar monofunctional complex *cis*-$[Pt(NH_3)_2$(4-methylpyridine)Cl$]^+$, which also forms monofunctional lesions at the d(GG) sites but with preference to their 5′ residues (Lempers *et al*., 1990).

The monofunctional adducts of cisPt-ACV inhibit DNA synthesis (Coluccia *et al*., 1995) and block specifically RNA polymerase at platinated guanine residues *in vitro* (Fig. 3). This observation is particularly interesting because the monofunctional lesions of several platinum(II) complexes are unable to terminate RNA synthesis by the RNA polymerase *in vitro* (Lemaire *et al*., 1991; Brabec and Leng, 1993; Brabec *et al*., 1994), whereas bifunctional adducts of all platinum compounds so far tested inhibit RNA synthesis at the level of these adducts (Lemaire *et al*., 1991; Anin *et al*., 1992; Brabec and Leng, 1993; Brabec *et al*., 1994). A question remains concerning the mechanism by which RNA synthesis is inhibited (i.e., in particular, what structural features of a monodentate fashion of DNA binding, previously expected not to result in inhibition of RNA synthesis, are required for blocking the RNA polymerase activity).

The results of immunochemical analysis (Fig. 9) demonstrate that some monofunctional lesions formed by cisPt-ACV at guanine residues in double-helical DNA inhibit the antibodies that specifically recognize and bind to intrastrand cross-links formed by cisplatin between neighboring purine residues. The latter observation is consistent with an idea that DNA adducts of cisPt-ACV have some structural motif similar to that produced by the major adduct of cisplatin (*vide supra*). We speculate that the ACV ligand itself interacts with DNA in a noncovalent manner, producing certain structural feature similar to the major adduct of cisplatin. These suggestions are corroborated by the observation that cisPt-ACV adducts destabilize DNA (Fig. 7) and affect conformation of DNA (Fig. 8) in ways that are qualitatively similar to the effects of bifunctional cisplatin and distinct from the effects of monofunctional, clinically ineffective dienPt. Thus, DNA adducts of cisPt-ACV could be recognized and further processed by some components of tumor cells (e.g., by some DNA or RNA polymerases) by a fundamentally different way than other monofunctional platinum adducts, including those of dienPt.

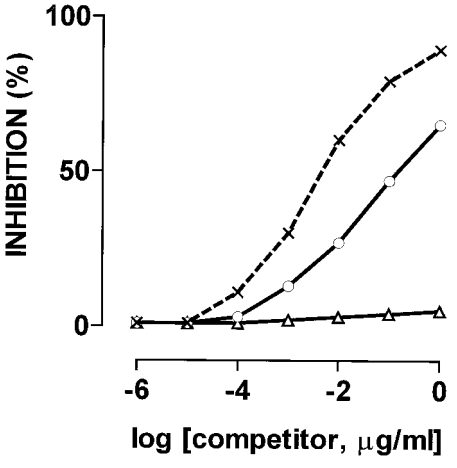

Fig. 9. Competitive inhibition in an ELISA of the cisplatin-DNA antibody binding (Ab_{cis}) to the competitor that was calf thymus DNA modified at r_b = 0.05 by either cisPt-ACV (○), cisplatin (×), or dienPt (△). The inhibition expresses the antibody binding as percentage of binding in the absence of any competitor. Data points measured in triplicate varied on average by ±2% from their mean.

Unique properties of DNA adducts of cisPt-ACV have been revealed in the current work. However, the fact that there are some similarities with the effects of antitumor cisplatin (terbium fluorescence, melting behavior, recognition by specific antibodies, termination of RNA synthesis) might be of a fundamental importance in explaining some aspects of anticancer activity of this new platinum(II)-triamine complex. On the other hand, the antitumor activity of cisPt-ACV is not identical with that exhibited by cisplatin and its bifunctional analogues (Coluccia *et al*., 1995). Thus, the results of this work support the view that the platinum drugs, which bind to DNA in a manner different from that of cisplatin and related analogues, can differ in activity profile. Further studies are in progress in our laboratories to reveal other details of the modification of DNA by cisPt-ACV most likely relevant to its cytostatic properties.

The current work has yielded new information on DNA interactions of the platinum(II)-triamine compounds. However, it also shows that cisPt-ACV modifies DNA in a way that is in some aspects similar to that of another *cis*-$[Pt(NH_3)_2(Am)Cl]^+$ complex (Hollis *et al*., 1991). It implies that the effect of the ACV ligand in the platinum-triamine complexes of this type on its DNA binding mode need not be strictly specific. The current work describes coordination of cisPt-ACV to DNA, consequences of this reaction for DNA conformation, and its processing by proteins (DNA or RNA polymerases and antibodies). However, this coordination of the drug to DNA must be involved, for obvious reasons, in the mechanism of antiviral activity of unplatinated nucleosides and their analogues such as unplatinated ACV. On the other hand, cisPt-ACV exhibits antiviral activity *in vitro* (Coluccia *et al*., 1995), so it cannot be excluded that at least some consequences of DNA interactions of cisPt-ACV revealed in the current work may play a role in the molecular mechanisms of antiviral activity of *cis*-$[Pt(NH_3)_2(Am)Cl]^+$ complexes, as they do in their antitumor effect. Further studies are therefore warranted to reveal the importance of DNA binding modes of the novel platinum(II) compounds containing, as a part of the coordination sphere, certain selected nucleosides for their antiviral efficiency.

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