Nucleic Acids Research, Vol. 20, No. 2 **267–272**

Biophysical analysis of DNA modified by 1,2-diaminocyclohexane platinum(II) complexes

Vladimír Boudný, Oldrich Vrána, Frederic Gaucheron[1], Vladimír Kleinwachter, Marc Leng[1] and Viktor Brabec*

Institute of Biophysics, Czechoslovak Academy of Sciences, Královopolská 135, 612 65 Brno, Czechoslovakia and [1]Centre de Biophysique Moléculaire, CNRS, 1A, Avenue de la Recherche Scientifique, 45071 Orléans Cedex 2, France

Received October 14, 1991; Revised and Accepted December 23, 1991

ABSTRACT

Modification of DNA and double-stranded deoxyoligonucleotides with antitumour 1,2-diaminocyclohexanedinitroplatinum(II) (Pt-dach) complexes was investigated with the aid of physico-chemical methods and chemical probes of nucleic acid conformation. The three Pt-dach complexes were used which differed in isomeric forms of the dach non-leaving ligand – Pt(1R,2R-dach), Pt(1S,2S-dach) and Pt(1R,2S-dach) complexes. The latter complex has lower antitumour activity than the other two Pt-dach complexes. Pt(1R,2S-dach) complex exhibits the slowest kinetics of its binding to DNA and of the conversion of monofunctional binding to bifunctional lesions. The anomalously slow electrophoretic mobility of multimers of the platinated and ligated oligomers suggests that bifunctional binding of Pt-dach complexes to a d(GG) site within double-stranded oligonucleotides induces bending of the oligomer. In addition, chemical probing of double-helical deoxyoligonucleotides modified by the Pt-dach complexes at the d(GG) sites reveals that Pt(1R,2S-dach) complex induces more extensive conformational changes in the oligomer than Pt(1R,2R-dach) and Pt(1S,2S-dach) complexes. It is proposed that different effects of the Pt-dach complexes on DNA observed in this work arise mainly from a steric crowding of the axially oriented cyclohexane ring in the DNA adduct of Pt(1R,2S-dach) complex.

INTRODUCTION

Platinum coordination complexes are widely used in the treatment of human cancer (1–4). Since the first platinum drug—cis-diamminedichloroplatinum(II) (cis-DDP)—was introduced in clinics, many platinum complexes have been synthesized and tested for anticancer activity.

Antitumour activity of platinum complexes is varied by the replacement of their leaving (5–8) and non-leaving (9–12) groups. Among the analogues of cis-DDP the platinum complexes which have 1,2-diaminocyclohexane (abbreviated as dach) as the non-leaving amine ligand (Fig. 1) have been especially noted as second generation of platinum drugs (1,9). It has been so also because they lack cross-resistance with established drugs like cis-DDP (13,14).

The ligand dach has three isomeric forms (Fig. 1A): the enantiomers Pt(1R,2R-dach) (trans-l-dach), Pt(1S,2S-dach) (trans-d-dach) and the diastereoisomer Pt(1R,2S-dach) (cis-dach). Generally, of the dach platinum compounds Pt(1R,2R-dach) and Pt(1S,2S-dach) complexes have a higher antitumour activity than Pt(1R,2S-dach) complex (4–6).

The interaction of platinum drugs with DNA has been implicated as essential to their antitumour effect (1–4). The adjacent guanine residues on the same DNA strand constitute a preferential platinum binding site in the reaction of bivalent platinum complexes having the leaving ligands in cis-configuration (2–4,15–17). The spectrum of adducts produced on DNA by the three Pt-dach complexes is similar for each isomer and similar to that reported for cis-DDP (16). Thus the simple analysis of the Pt-dach adducts does not provide an explanation of the different antitumour activities of the isomers.

As has been discussed by Kidani et al. (9) the 1,2-diaminocyclohexane ligand in the bifunctional adducts of Pt(1R,2R-dach) or Pt(1S,2S-dach) complexes at the d(GG) sites of DNA adopts a configuration, in which the plane of the cyclohexane ring is almost parallel with the coordination plane in the planar bivalent platinum complexes. It means that the cyclohexane ring is roughly perpendicular to the helix axis of DNA so that the relatively bulky non-leaving diaminocyclohexane ligand can be accomodated in the major groove of DNA without a significant steric hindrance. A somewhat different situation is in the case of reaction of DNA with the Pt(1R,2S-dach) complex. In the latter complex one amino group is bound to the cyclohexane ring axially and the other equatorially (Fig. 1A). Thus two configurations of 1,2-diaminocyclohexane ligand can exist in the DNA adducts of Pt(1R,2S-dach) complex (Fig. 1B) that are mutually interchangeable with the simultaneous inversion of

* To whom correspondence should be addressed

cyclohexane ring. The cyclohexane ring of Pt(1R,2S-dach) complex in both adducts is oriented almost perpendicularly to the coordination plane of the bivalent platinum. The theoretical analysis reveals (9) that steric crowdings are minimum for the formation of the bifunctional adduct with the cyclohexane ring oriented on the side oposite to the location of O(6) atoms of guanine residues [Pt(1R,2S-dach)-DNA(I) adduct, Fig. 1B, left]. On the other hand, the origin of the Pt(1R,2S-dach)-DNA(II) adduct with the cyclohexane ring oriented on the side of the O(6) atoms (Fig. 1B, right) should induce further alterations in the conformation in the regions of DNA molecule close to the d(GG) site to which Pt(1R,2S-dach) complex is bound.

The goal of this work was to establish whether the steric structure of the non-leaving group of platinum dach complexes could influence on one hand the binding of the drugs to DNA and on the other hand the distortions induced in the double helix of DNA.

MATERIALS AND METHODS

The platinum dach-complexes {cis-Pt[dach$(NO_3)_2$]} were kindly provided by Professor Y.Kidani (Nagoya, Japan). Cis-DDP and cis-dichloro(ethylenediamine)platinum(II) were from Lachema (Brno, Czechoslovakia). The platinum complexes were dissolved in 10 mM $NaClO_4$ and used immediately. Calf-thymus DNA was isolated and characterized as described in our previous papers (18,19). Thiourea randomly labeled with ^3H was purchased from the Institute for Research, Development and Application of Radioisotopes in Prague and nonradioactive thiourea from Lachema, Brno.

The kinetics of reaction of the platinum complexes with double-helical DNA were measured with the aid of differential pulse polarography (20). Since the polarographic technique determines the concentration of unbound platinum in the reaction mixture the kinetic curves correspond to the initial (monofunctional) binding step.

Thiourea binding assay was used to determine kinetics of the rearrangement of monofunctional adducts of platinum compounds into the bifunctional ones (15,21). Platinum complex at the concentration of 4 μM was mixed with a solution of DNA at the concentration of 0.16 mg/ml (number of platinum atoms added to the reaction mixture with DNA per one nucleotide residue, r_i, was 0.08) in 10 mM $NaClO_4$ at 28°C. After 20 minutes the initial mono functional reaction was stopped by separating the unbound platinum complex with the aid of a 30 s centrifugation at 1500 rpm through a column of Sephadex G25 (coarse). The amount of bound platinum per nucleotide (r_b) at the time of separation was determined polarographically in a parallel experiment. After the separation aliquots of 0.15 ml were withdrawn at various time intervals and added to 0.15 ml of ^3H labeled thiourea. The tritiated thiourea solution was prepared by 1:1 mixing the stock solution of 1.73 mM [^3H] thiourea having a specific activity of 1850 MBq/mmol with 7.23×10^{-5} M non-radioactive thiourea. After 10 min incubation at 25°C, 0.8 ml of 0.15 M NaCl, pH 7.0 was added and 1.0 ml of the resulting solution was layered on a nitrocellulose filter having pores of 0.4 mm in diameter (Synpor, VCHZ Synthesia, Pardubice). In order to remove the unreacted thiourea the filter was washed with 15 ml of 5 % trichloroacetic acid. The filters were dried under an infrared lamp, transferred to glass tubes to which 5 ml of toluene scintillator was added. The radioactivity was measured on a Pharmacia Wallac 1410 Liquid Scintillation Counter (2×2 min). The content of the platinum bound to DNA monodentately was calculated using a suitable calibration curve.

Semilogarithmic plots of the kinetic data (a decrease of concentration of free platinum in the pulse-polarographic experiments or a decrease of platinum atoms available for the reaction with thiourea) could be resolved into two linear parts corresponding to two parallel reactions. Pseudo-first order rate constants were determined from the slopes of linear fits to the semilogarithmic plots. The slope for the fast reaction was obtained by subtracting the line corresponding to the slower reaction.

Other details of physico-chemical measurements were described earlier (20,22,23).

Deoxyribooligonucleotides d(CTTCTCTTCTGGTCTTCTCT) and d(AAGAGAAGACCAGAAGAGAG) [abbreviated respectively d(TGGT) and d(ACCA)] were synthesized on an Applied Biosystem solid-phase synthesizer. They were purified in two steps by ion-exchange and reverse-phase HPLC (Hitachi Model 655 HPLC). The oligonucleotide d(TGGT) reacted with the platinum complexes in 10 mM $NaClO_4$ at 37°C in the dark for 48 hours. The platinated oligonucleotides were again purified in two steps by ion-exchange and reverse-phase HPLC. The major product, d(TGGT) modified bidentately at the d(GG) site by cis-DDP or the platinum dach-complexes [designated as d(TG*G*T)], were shown by atomic absorption spectroscopy to have about 1 platinum atom per oligonucleotide molecule. The sites of platination were verified by HPLC as described in the earlier report (24). The yields of d(TG*G*T) were about 60%. Unplatinated d(ACCA) in the experiment in which diethylpyrocarbonate (DEPC) was used as a chemical probe and d(TG*G*T) were labeled at the 5′ end with [gamma-^{32}P]ATP by means of T4 polynucleotide kinase. They were then hybridized with the complementary strand.

The chemical probing of platinated oligonucleotide duplexes was performed as described by Herr (25) for DEPC and by Nejedly et al. (26) for osmium tetraoxide. Piperidine treatment and preparation of the samples were identical as for sequencing reactions (27). Equivalent amounts of radioactivity for each sample were loaded on a 20% polyacrylamide sequencing gel. Chemical degradation sequencing reactions were as described earlier (27).

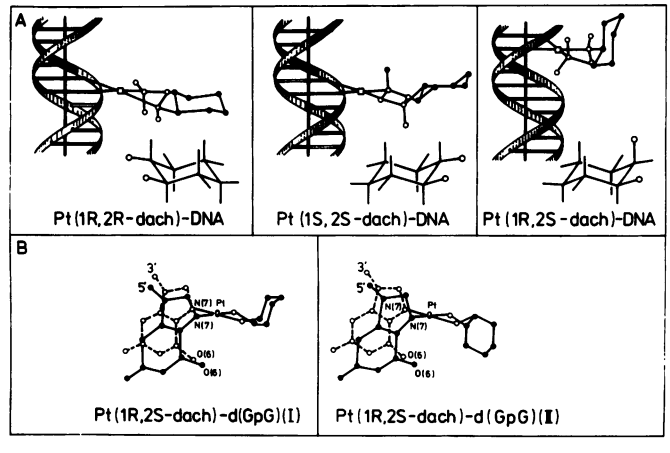

Fig. 1. A (upper row): Schematic structure of DNA adducts of platinum-dach complexes. B (lower row): Schematic structure of the two DNA adducts of Pt(1R,2S-dach) complex.

The chemical probing of the conformation of the platinated oligonucleotide duplexes with the aid of sodium cyanide was performed as described by Schwartz et al. (28). The oligonucleotide duplexes treated with 0.2 M NaCN in 20 mM Tris.HCl, pH 8.3 and control untreated samples were run on a denaturing 24% polyacrylamide gel.

The hybridized duplexes were self-ligated to make multimers of the oligonucleotide according to the procedure described by Koo et al. (29). The ligated products were run on 8% polyacrylamide gels [mono:bis(acrylamide)] ratio was 29 : 1; the medium was 90 mM Tris borate, pH 8 with 2.5 mM EDTA. The applied voltage was 7 V/cm, and electrophoresis was performed at room temperature.

Electrophoresis-grade acrylamide and bis(acrylamide) were from Merck (FRG).

RESULTS AND DISCUSSION

The kinetics of the binding of Pt-dach complexes to calf-thymus DNA

Kinetic curves of the reaction between the three Pt[$(NO_3)_2$dach] complexes (Fig. 1) and double-helical calf thymus DNA (the input ratio of molar concentrations of a platinum complex and nucleotide residues, r_i, was 0.08) in the medium of 10 mM $NaClO_4$ at 28°C were obtained by determining the decrease of free platinum concentration by means of differential pulse polarography (20). These measurements do not differentiate between monofunctional and bifunctional binding of the platinum complexes and monitor only the initial reaction step. A semilogarithmic plot of concentration of the free Pt(1R,2R-dach) complex versus time is shown in Fig. 2A. It can be resolved into two linear parts, which are assumed to correspond to two parallel pseudo-first-order reactions. The same shape but different values were obtained for polarographic binding curves of the two other Pt-dach complexes. Apparent rate constants calculated from the slopes of the binding curves are given in Table 1.

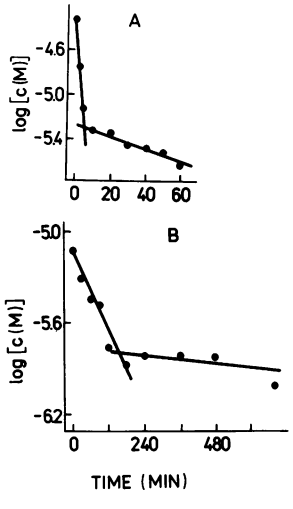

Fig. 2. A: A semilogarithmic plot of the concentration c of free Pt(1R,2R-dach) complex on time during the reaction of this platinum complex with DNA. B: The kinetic study of conversion of the monofunctional binding of Pt(1R,2R-dach) complex to DNA to bifunctional attachment performed with the aid of thiourea binding assay. A semilogarithmic plot of the concentration of monofunctionally fixed Pt(1R,2R-dach) complex on time is shown. The details of the experiments shown in this figure are described in the sections Materials and Methods and Results of this paper.

The biphasic character of the reactions is similar to the kinetics observed for the binding of other platinum compounds to DNA (20,23) and indicates that the reactive sites on the DNA molecule exhibit different affinity towards the dach platinum complexes. The apparent rate constants of the three dach platinum complexes (Table 1) exhibit some correlation with their biological activity. The initial binding is slowest for Pt(1R,2S-dach) which exhibits the lowest anticancer activity.

Conversion of monofunctional calf-thymus DNA adducts of Pt-dach complexes to bifunctional lesions

The conversion of the monofunctional binding to bifunctional attachment was studied with the aid of thiourea binding assay (15,21). During the initial period of the reaction of DNA with bifunctional platinum complexes, when a significant part of the molecules is bound monofunctionally, the other coordination site can be blocked by thiourea, since sulphur has a higher stability constant than heterocyclic nitrogen (21). On the basis of this phenomenon the rate of quenching of the monofunctional adducts, i.e. the rate of formation of the bifunctional ones, may be measured. The kinetic measurements were carried out in 10 mM $NaClO_4$ at 28°C with samples, from which the unbound platinum complex was removed by centrifugation through a Sephadex column 20 minutes after the start of the reaction. The input ratio r_i was always 0.08 at DNA concentration of 0.16 mg/ml; after the separation of free platinum molar ratios of covalently bound platinum atoms per nucleotide residue, r_b, were 0.078, 0.077, and 0.073 for Pt(1R,2R-dach), Pt(1S,2S-dach), and Pt(1R,2S-dach) complexes, respectively. After 20 minutes of the reaction of Pt(1R,2R-dach), Pt(1S,2S-dach) and Pt(1R,2S-dach) complexes with DNA 23, 13 and 37% of the adducts were monofunctional lesions, respectively. The semilogarithmic plots of the concentration of the platinum complex bound to DNA monodentately on time had a biphasic character [the plot obtained for Pt(1R,2R-dach) complex is shown in Fig. 2B as an example]. The apparent first-order rate constants (Table 2) were calculated under assumption that only one molecule of thiourea is bound to one platinum complex fixed to DNA monofunctionally. The apparent rate constant calculated for the bifunctional rearrangement of Pt(1R,2S-dach) complex is significantly lower than the values obtained for the reactions of Pt(1R,2R-dach) and Pt(1S,2S-dach) complexes. The apparent

Table 1. Apparent rate constants (k_{app}) for reaction of DNA with platinum dach-complexes determined polarographically

Ligand	$k_{app}(1)$ (min^{-1})	$k_{app}(2)$ (min^{-1})
Pt(1R,2S-dach)	0.283	0.017
Pt(1R,2R-dach)	0.549	0.012
Pt(1S,2S-dach)	0.345	0.005

Table 2. Apparent rate constants K_{app} of monoadduct quenching during reaction of platinum dach-complexes with DNA determined by thiourea assay

Ligand	$K_{app}(1)$ (min^{-1})	$K_{app}(2)\times10^4$ (min^{-1})
Pt(1R,2S-dach)	0.006	2
Pt(1R,2R-dach)	0.017	5
Pt(1S,2S-dach)	0.012	5

rate constants of the slower phase were by more than one order of magnitude lower, but the difference between Pt(1R,2S-dach) on the one hand and Pt(1R,2R-dach) and Pt(1S,2S-dach) complexes on the other remained preserved.

The kinetic measurements carried out by means of thiourea reveal that the rearrangement of monofunctional binding of dach-complexes to DNA to bifunctional adducts is slowest in the case of the Pt(1R,2S-dach) complex. We propose that this observation arises from a steric crowding of the axially oriented cyclohexane ring in the Pt(1R,2S-dach)-DNA(II) adduct (9) (Fig. 1 B).

Electrophoretic mobility of multimers of 20 bp oligomers modified by Pt-dach complexes

The results described above were obtained with high-molecular mass mammalian DNA that contained different types of Pt-dach adducts. More precise conclusions on conformational distortions induced in DNA by platinum complexes can be obtained with oligonucleotides of defined sequence containing only one type of platinum adduct. Therefore, the further experiments were carried out with double-helical 20-mers named d(TG*G*T).d(ACCA) modified bidentately at the d(GG) sites with the Pt-dach complexes and for comparison also with cis-DDP and cis-dichloro(ethylenediamine)platinum(II) [cis-(en)Pt]. The latter platinum complex is used instead of cis-DDP if radiolabeling of the platinum complex is required (17).

Intrinsic bending of DNA duplexes results in the abnormal electrophoretic mobility of DNA fragments (30−34). A gel migration anomaly has been found for DNA fragments containing bidentate adducts formed by cis-DDP at the d(GG), d(AG) and d(GTG) sites (24,34−38). As shown in Fig. 3, a retardation in gel mobility occurs with oligonucleotide multimers prepared by ligation of oligonucleotides [d(TG*G*T).d(ACCA)] containing bidentate intrastrand adducts formed by all platinum complexes used throughout this work. This is also illustrated by the value of the K-factor (ratio of apparent length to true length), which is equal for the sequence length of 160 bp to 1.56 for cis-DDP, 1.52 for cis-(en)Pt and Pt(1R,2R-dach) complex and 1.36 for Pt(1S,2S-dach) complex. It is known that cis-DDP bound at the d(GG) site bends DNA (34). Our results suggest that cis-DDP, cis-(en)Pt and Pt-(1R,2R-dach) complex bound to the d(GG) site bend DNA similarly while Pt(1S,2S-dach) complex is less effective. As already mentioned above Pt(1R,2S-dach) complex is expected to form on DNA two different bifunctional adducts in the d(GG) sites. Pt(1R,2S-dach)-DNA(I) adduct (Fig. 1B) should induce the distortion which is similar to that induced by cis-DDP or Pt(1R,2R-dach) or Pt(1S,2S-dach) complexes. The other adduct [Pt(1R,2S-dach)-DNA(II), Fig. 1B] is expected to induce the conformational alterations that would be more extensive. It is reasonable to expect that the latter distortion results in somewhat different (presumably more pronounced) bending of DNA double helix [in comparison with Pt(1R,2S-dach)-DNA(I) adduct, Fig. 1B] and consequently in a different electrophoretic migration of the multimers. In other words the sample containing multimers of oligonucleotides modified by Pt(1R,2S-dach) complex should contain chains formed by ligation of the two types of duplexes that differ in the magnitude of the bending at the site of platination. It is apparent that these multimers will not yield sharp electrophoretic bands. For instance already in the case of a dimer theoretically three molecules of the same sequence may origin that differ in the types of Pt(1R,2S-dach)--DNA adduct and, consequently, in the apparent length measured with the aid of electrophoresis in non-denaturing polyacrylamide gel. The variety in the population of the molecules formed by the ligation that differ in the apparent length but have the same sequential length and thus also in the electrophoretic mobility should increase with the growing sequential length of multimers. This increasing variety should result in a broadening of electrophoretic bands and in a lowering of their intensity. The result obtained (the lane 4 in Fig. 3) corresponds to the foregoing analysis. Thus Pt(1R,2S-dach) complex induces in the d(GG) sites of DNA two types of the bifunctional adducts that differ in the extent of distortions in the DNA double helix.

Chemical probing of conformational distortions induced in 20 bp oligomers by Pt-dach complexes

It has been suggested (9) that the formation of Pt(1R,2S-dach)-DNA(II) adduct (Fig. 1B) results in a displacement of some

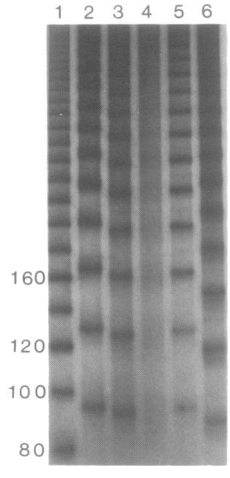

Fig. 3. Comparison of the migration of platinated 20 bp oligomers d(TG*G*T)+d(ACCA) ligated to multimers on a nondenaturing 8% polyacrylamide gel. Unplatinated oligomers d(TGGT)+d(ACCA) ligated to multimers (lane 1); the oligomers modified with: cis-DDP (lane 2), cis-dichloro(ethylenediamine) platinum(II) (lane 3), Pt(1R,2S-dach) complex (lane 4), Pt(1R,2R-dach) complex (lane 5), Pt(1S,2S-dach) complex (lane 6).

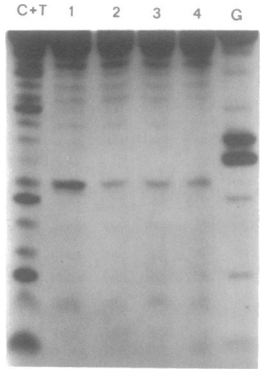

Fig. 4. Piperidine-induced specific strand cleavage at OsO_4 modified bases in platinated 20 bp oligonucleotides d(TG*G*T)+d(ACCA) (lanes 1−4). OsO_4 reacted with the platinated oligomer d(TG*G*T) in the duplex d(TG*G*T)+d(ACCA). Maxam-Gilbert specific reactions T+C (lane T+C) and G (lane G) for the unplatinated oligonucleotides are also given. (Lane 1) Pt(1R,2S-dach)-modified oligomer; (lane 2) Pt(1S,2S-dach)-modified oligomer; (lane 3) Pt(1R,2R-dach)-modified oligomer; (lane 4) cis-DDP-modified oligomer.

adjacent groups of the sugar-phosphate backbone of DNA from their position given by the geometry of B-DNA. We used OsO_4 and DEPC as chemical probes of DNA conformation to verify this suggestion. After reaction with the chemical probes, the labeled platinated oligonucleotide duplexes were incubated with NaCN to remove bound platinum (39) and were then treated with piperidine. The resulting fragments were resolved on a sequencing gel along with the Maxam-Gilbert sequencing ladders of the same unplatinated oligonucleotide duplex.

In the oligonucleotide duplex d(TG*G*T).d(ACCA) platinated with Pt(1R,2S-dach) complex the thymine residue on the 5′ side of the d(GG) site reacts with OsO_4 (Fig. 4). On the other hand, this residue reacts only negligibly with OsO_4 if the oligonucleotide duplex is bifunctionally modified by cis-DDP, Pt(1R,2R-dach) or Pt(1S,2S-dach) complexes. DEPC, which is a probe of unpaired adenine residues (25,33), does not react with adenine residue complementary to thymine residue reactive with OsO_4 even in the oligonucleotide duplex modified with Pt(1R,2S-dach) complex. It has been verified that the single-stranded oligonucleotides are reactive with the chemical probes, while unplatinated double-helical oligonucleotides are not reactive. In summary, DNA modified only by the Pt(1R,2S-dach) complex is sensitive to the chemical probe OsO_4, which suggests a larger distortion of the double helix induced by this compound as compared to the other platinum derivatives used throughout this work.

The view that the binding of Pt(1R,2S-dach) complex to DNA induces in this biomacromolecule more extensive conformational alterations is also supported by the experiments in which sodium cyanide was used as a probe of the DNA conformation modified by the platinum complexes (28). Cyanide ions can rapidly remove cis-DDP from the double-helical oligonucleotides modified by this platinum complex at the d(GG) sites. It has been shown that the kinetics of the reaction between cyanide ions and the d(GG) bifunctional adduct of cis-DDP is strongly dependent upon the DNA conformation. The samples of d(TG*G*T).d(ACCA) modified by cis-DDP and all three platinum dach complexes (c. 3×10^{-6} M) were treated with a large excess of cyanide ions. At various times, aliquots were withdrawn and analyzed by gel electrophoresis under denaturing conditions (28). The half-lives of the disappearance of the starting products [the oligonucleotides modified bidentately at the d(GG) site with Pt(1R,2R-dach), Pt(1S,2S-dach) and Pt(1R,2S-dach) complexes] are 40, 60, and 100 minutes, respectively. Thus cyanide ions are more reactive with the double-helical oligonucleotide modified by Pt(1R,2R-dach) and Pt(1S,2S-dach) complexes as compared with the oligomer modified by Pt(1R,2S-dach) complex. It has been shown (28) that the rate of removal of the bound platinum residues decreases when the distortion induced by the Pt-d(GG) adduct is larger. Thus probing the conformation of DNA with sodium cyanide confirms larger distortion induced in DNA by the binding of Pt(1R,2S-dach) complex in comparison with Pt(1R,2R-dach) and Pt(1S,2S-dach) complexes.

CONCLUSIONS

Taken together the results of this work demostrate that the binding of cis-DDP analogues to DNA and resulting distortions of DNA double helix are dependent on the structure of non-leaving ligands of platinum complexes. So far pharmacological structure-activity relationships were mainly observed for antitumour cis-DDP and its inactive trans-isomer. This paper shows that this relationship can be also observed on the basis of even more subtle differences in the structure of the platinum complexes and their DNA adducts than in the case of cis-DDP and its trans-isomer. It has to be, however, emphasized that the pharmacological structure-activity relationship observed on the level of reactions of platinum complexes with DNA *in vitro* does not entirely explain the mechanism of antitumour activity of platinum complexes. There are still many biochemical processes with which platinum complexes may interfere in cancer cells to induce cytostatic effect. Nevertheless, this paper supports the view that among these biochemical events the reaction of platinum drugs with DNA is crucial.

ACKNOWLEDGEMENT

The assistance of Dr. Marie Vojtisková in using chemical probes of DNA conformation is gratefully acknowledged.

REFERENCES

1. Cleare,M.J., Hydes,P.C., Hepburn,D.R. and Malerbi,B.W. (1980) Cisplatin, Prestayko,A.W., Crooke,S.T. and Carter,S.K., Eds, Academic, New York, p. 149–170.
2. Lepre,C.A. and Lippard,S.J. (1990) Nucleic Acids and Molecular Biology, Eckstein,F. and Lilley,D.M.J., Eds, Springer-Verlag, Berlin, **4**, p. 9–38.
3. Reedijk,J. (1987) Pure and Appl Chem. **59**, 181–192.
4. Johnson,N.P., Butour.,J.-L., Villani,G., Wimmer,F.L., Defais,M., Pierson,V. and Brabec,V. (1989) Prog. Clinical Biochem. Med., Springer-Verlag, Berlin, **10**, p. 1–24.
5. Cleare,M.J., Hydes,P.C., Malerbi,B.W. and Watkins,D.M. (1978) Biochimie **60**, 835–850.
6. Al-Sarraf,M., Kish,J., Ensley,J., Metch,B., Rinehart,J., Schuller,D. and Coltman,C. (1987) Proc. Am. Soc. Clin. Oncol. **6**, A485.
7. Pendyala,L., Arakali,A.V., Sansone,P., Cowens,J.W. and Creaven,P.J. (1990) Cancer Chemother. Pharmacol. **24**, 248–250.
8. Alberts, D.S., Young,L. and Salmon,S.E. (1986) Proc. Am. Soc. Clin. Pharmacol. Ther., p.2.
9. Inagaki,K. and Kidani,Y. (1986) Inorg. Chem. **25**, 1–3.
10. Kidani,Y. and Inagaki,K. (1978) J. Med. Chem. **21**, 1315–1318.
11. Noji,M., Otamato,K. and Kidani,Y. (1981) J. Med. Chem. **24**, 508–515.
12. Vollano,J.F., Al-Baker,S., Dabrowiak,J.C. and Schurig,J.E. (1987) J. Med. Chem. **30**, 716–719.
13. Burchenal,J.H., Kalaher,K., O'Toole,T. and Chisholm,J. (1977) Cancer Res. **37**, 3455–3457.
14. Burchenal,J.H., Kalaher,K., Dew,K., Lokys,L. and Gale,G. (1978) Biochimie **60**, 961–965.
15. Page,J.D., Husain,I., Sancar,A. and Chaney,S.G. (1990) Biochemistry **29**, 1016–1024.
16. Jennerwein,M.M., Eastman,A. and Khokhar,A. (1989) Chem.-Biol. Interact. **70**, 39–49.
17. Eastman,A. (1987) Pharmacol. Ther. **34**, 115–166.
18. Brabec,V. and Palecek,E. (1970), Biophysik **6**, 290–300.
19. Brabec,V. and Palecek,E. (1978), Biophys. Chem. **4**, 76–92.
20. Kim.,M.H., Vrana,O., Kleinwachter,V., Niki,K. and Brabec,V. (1990) Anal.Letters **23**, 1505–1518.
21. Eastman,A. (1986) Biochemistry **25**, 3912–3915.
22. Vrána,O., Brabec,V. and Kleinwachter,V. (1986) Anti-Cancer Drug Design **1**, 95–109.
23. Kleinwachter,V., Brabec,V., Vra 'na,O. and Johnson,N.P. (1988) studia biophys. **123**, 85–93.
24. Marrot,L. and Leng,M. (1989) Biochemistry **28**, 1454–1461.
25. Herr,W. (1985) Proc.Nat.Acad.Sci.USA **82**, 8009–8013.
26. Nejedlý,K., Kwinkowski,M., Galazka,G., Klysik,J. and Palecek,E. (1985) J.Biomol.Struct.Dyn. **3**,467–478.
27. Maxam,A.M. and Gilbert,W. (1980) Methods Enzymol. **65**, 499–560.
28. Schwartz,A., Sip,M. and Leng,M. (1990) J. Amer. Chem. Soc. **112**,3673–3674.
29. Koo,H., Wu,H.M. and Crothers,D.M. (1986) Nature (London) **321**,501–506.

30. Diekman,S.V. (1987) Nucleic Acids and Molecular Biology, Eckstein,F. and Liley,D.M.J., Eds, Springer, Berlin, **1**, p. 138–156.
31. Trifonov,E.N. and Ulanovsky,L.E. (1988) Unusual DNA Structures, Wells,R.D. and Harvey,S.C., Eds, Springer, Berlin, p. 225–236.
32. Hagerman,P.J. (1988) Unusual DNA Structures, Wells,R.D. and Harvey,S.C., Eds, Springer, Berlin, p. 225–236.
33. Leng,M. (1990) Biophys. Chem. **35**, 155–163.
34. Rice,J.A., Crothers,D.M., Pinto,A.L. and Lippard,S.J. (1988) Proc.Nat.Acad.Sci. USA **85**, 4158–4161.
35. Schwartz,A., Marrot,L. and Leng,M. (1989) Biochemistry **28**, 7975–7979.
36. Bellon,S.F. and Lippard,S.J. (1990) Biophys. Chem. **35**, 179–188.
37. Bellon,S.F., Coleman,J.H. and Lippard,S.J. (1991) Biochemistry **30**, 8026–8035.
38. Anin,M.-F. and Leng,M. (1990) Nucleic Acids Res. **18**, 4395–4400.
39. Bauer,W., Gonias,S.L., Kam,S.K., Wu,K.C. and Lippard,S.J. (1978) Biochemistry **17**,1060–1068.