Biophysical Chemistry, 35 (1990) 179–188
Elsevier

BIOCHE 01422

Bending studies of DNA site-specifically modified by cisplatin, *trans*-diamminedichloroplatinum(II) and *cis*-[Pt(NH_3)$_2$(*N*3-cytosine)Cl]$^+$

Steven F. Bellon and Stephen J. Lippard

Department of Chemistry, Massachussetts Institute of Technology, Cambridge, MA 02139, U.S.A.

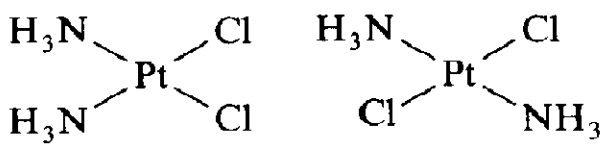
Received June 1989

cis-Diamminedichloroplatinum(II); DNA bending; Oligonucleotide hinge joint

Duplex oligonucleotides containing a single intrastrand {Pt(NH_3)$_2$}$^{2+}$ cross-link or monofunctional adduct and either 15 or 22 bp in length were synthesized and chemically characterized. The platinum-modified and unmodified control DNAs were polymerized in the presence of DNA ligase and the products studied on 8% native polyacrylamide gels. The extent of DNA bending caused by the various platinum-DNA adducts was revealed by their gel mobility shifts relative to unplatinated controls. The bifunctional adducts *cis*-[Pt(NH_3)$_2${d(GpG)}]$^+$, *cis*-[Pt(NH_3)$_2${d(ApG)}]$^+$, and *cis*-[Pt(NH_3)$_2${d(G*pTpG*)}], where the asterisks denote the sites of platinum binding, all bend the double helix, whereas the adduct *trans*-[Pt(NH_3)$_2${d(G*pTpG*)}] imparts a degree of flexibility to the duplex. When modified by the monofunctional adduct *cis*-[Pt(NH_3)$_2$(*N*3-cytosine)(dG)]Cl the helix remains rod-like. These results reveal important structural differences in DNAs modified by the antitumor drug cisplatin and its analogs that could be important in the biological processing of the various adducts in vivo.

1. Introduction

cis-Diamminedichloroplatinum(II), cisplatin or *cis*-DDP, currently used widely in cancer chemotherapy, is thought to act by binding DNA and



cis-DDP *trans*-DDP

preventing replication. The *trans* isomer as well as monofunctional platinum complexes such as [Pt(NH_3)$_3$Cl]Cl and [Pt(diethylenetriamine)Cl]Cl are inactive. These structure-function comparisons led to the conclusion that two labile groups and two *cis* ammine ligands were required for biological activity [1]. Recently, a new class of platinum-based antitumor drugs has been discovered in which platinum has only one labile ligand [2]. These compounds have two *cis* ammines, an anionic leaving group, and a heterocyclic pyridine, pyrimidine, purine or piperidine ligand [2]. They bind DNA monofunctionally without loss of ammonia or the heterocycle [3b], thus violating the traditional rules used to predict the activity of platinum antitumor drugs.

Replication mapping studies revealed that bifunctional adducts formed by *cis*- and *trans*-DDP, and monofunctional adducts afforded by the new class of antitumor active compounds, are all capable of blocking DNA replication, whereas adducts of the inactive monofunctional complexes [Pt(NH_3)$_3$Cl]Cl and [Pt(dien)Cl]Cl could not [3]. Knowledge of DNA structural changes that accompany platinum binding is required to understand replication inhibition and the differential processing of DNA adducts such as occurs in

Correspondence address: S.J. Lippard, Department of Chemistry, Massachussetts Institute of Technology, Cambridge, MA 02139, U.S.A.

0301-4622/90/$03.50 © 1990 Elsevier Science Publishers B.V. (Biomedical Division)

DNA repair [4,5]. One potentially important such structural motif is DNA bending [6,7].

The question of platinum-induced DNA bending has been addressed in previous structural work. Single-crystal X-ray diffraction studies of *cis*-[$Pt(NH_3)_2${d(pGpG)}] [8] and *cis*-[$Pt(NH_3)_2$-{d(CpG*pG*)}] [9] revealed that platinum binding destacks adjacent guanines, resulting in dihedral angles between ring planes of 76–87°. A kink in the helix axis may occur when the surrounding base-pairs stack on the perturbed guanines. Molecular mechanics calculations on duplex oligomers containing the *cis*-[$Pt(NH_3)_2$-{d(pGpG)}] intrastrand cross-link (*cis*-GG) revealed that structures kinked by about 60°, as well as unbent DNA molecules, are energetically feasible [10]. From gel electrophoresis mobility shifts the *cis*-GG bend angle was estimated to be about 40° [11]. Recently, the same assay was employed to examine oligomers containing the adducts *cis*-[$Pt(NH_3)_2${d(pApG)}] (*cis*-AG) and *cis*[$Pt(NH_3)_2${d(G*pTpG*)}] (*cis*-GTG). Both exhibited anomalous gel mobility shifts attributed to DNA bending [12]. Time-dependent fluorescence depolarization studies of calf thymus DNA globally platinated with *cis*- or *trans*-DDP were interpreted as evidence that DNA bends only when the former, but not the latter, is bound [13].

In the present work, we have extended the gel mobility shift methodology [6,11,12,14–17] to measure the bending of several site-specifically platinated oligomers. In particular, specific platinum-DNA adducts have been uniquely incorporated into double-stranded 22 bp oligodeoxyribonucleotides (fig. 1). Bend angle measurements [11] on *cis*-GG, comprising about 65% of all *cis*-DDP-DNA adducts [18–20], have been refined. The next most common adduct, *cis*-AG, accounting for another 25% of platinum bound to DNA [18–20], has also been investigated. Although adducts formed by *trans*-DDP are less well understood [21], 1,3-intrastrand d(GNG) cross-links probably comprise a significant fraction of the total [21]. DNA bending caused by the adduct *trans*-[$Pt(NH_3)_2${d(G*pTpG*)}], or *trans*-GTG, was therefore investigated. Finally, we have extended our studies to an active triammine complex, viz., *cis*-[$Pt(NH_3)_2$(*N*3-cytosine) Cl]Cl, or N3-C, monoadducted to a single guanosine, (N3-C)-G. We have also examined an oligonucleotide containing the *cis*-GTG intrastrand cross-link, for comparison with 1,3 adducts made by *trans*-DDP.

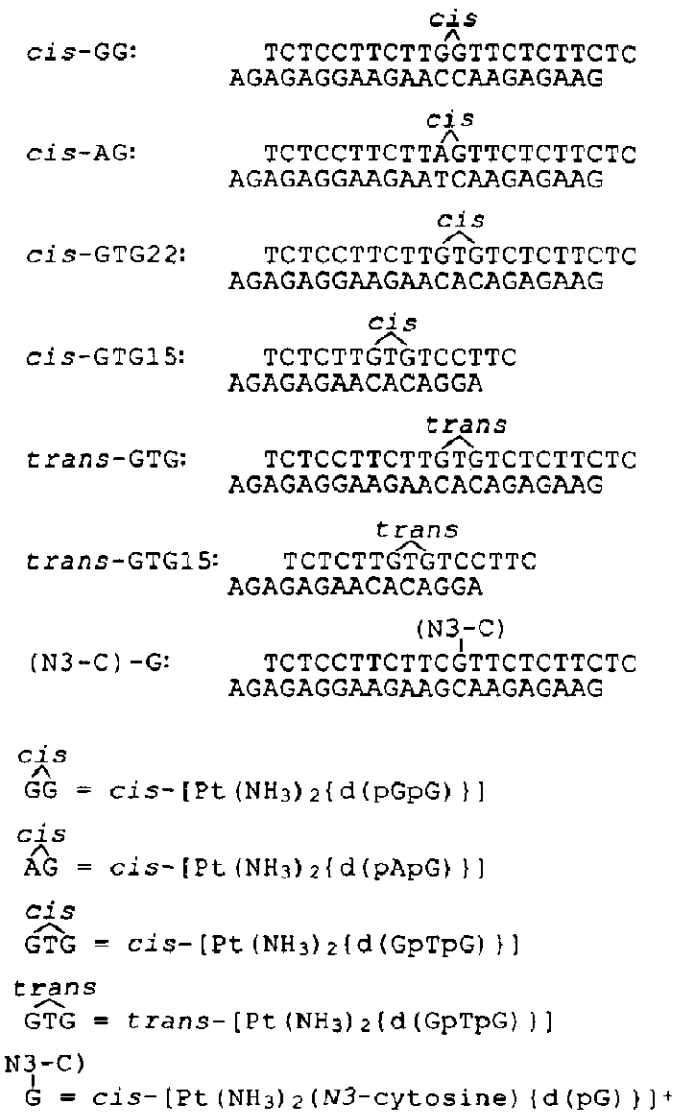


Fig. 1. Sequences used in bending experiments. *cis*-GG, *cis*-AG, *cis*-GTG, *trans*-GTG, and (N3-C)-G, are used in this paper to refer either to the adduct or the specific oligonucleotide shown, as will be clear from the reference context.

2. Materials and methods

Sequences used to study DNA bending and their abbreviations are shown in fig. 1. Oligomers were prepared on a Biosearch model 8600 DNA synthesizer in 1 μmol quantities. Workup consisted of deprotection with 2 ml of NH_4OH at 55°C for 12 h, separation from cleaved protecting groups on a Sephadex G-10 column, and purification from failed sequences by using reversed-phase

C-18 HPLC with isocratic flow (Waters Radial-Pak Cartridge, no. 85721). Oligomers were converted to their sodium salts on a Dowex cation-exchange column prior to phosphorylation or platination.

Platination of single-stranded, purified oligomers was carried out using 100–300 μM DNA at a formal platinum/DNA strand ratio (r_f) of 1.1–1.5. *cis*-DDP was allowed to react with 1.97 equiv. of $AgNO_3$ to form *cis*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ prior to reaction with DNA. *trans*-DDP and *cis*-$[Pt(NH_3)_2(N3\text{-Cyt})Cl]Cl$ were used in their chloride forms. In all reactions, the major product was isolated and separated from the reaction mixture by reversed-phase HPLC.

Platinum-DNA adducts were characterized by several methods. The purity of all oligomers was assessed by HPLC and on 8% native polyacrylamide electrophoresis gels. Carbon rod atomic absorption spectroscopy was used to determine the ratio of bound platinum per strand of DNA. Finally, all oligomers were digested to nucleosides in order to establish base composition by using DNase I (Sigma), P_1 nuclease, and alkaline phosphatase (both from Boehringer Mannheim) according to the method of Eastman [19]. Approx. 20 μg of DNA was digested [19] in 120 μl at 37 °C for 24 h, the products were separated by reversed-phase HPLC (5–22.5% CH_3CN in 30 min gradient at 1 ml/min flow), and the peaks were identified by coinjection with model compounds. *cis*-$[Pt(NH_3)_2\{d(GpG)\}]^+$ and *trans*-$[Pt(NH_3)_2(dG)_2]^{2+}$ were gifts from W.I. Sundquist and C.A. Lepre, respectively, in our laboratory. *cis*-$[Pt(NH_3)_2\{d(ApG)\}]^+$ and *cis*-$[Pt(NH_3)_2(N3\text{-cytosine})(dG)]^{2+}$ were prepared and characterized with assistance from W.I. Sundquist and K. Ahmed. All model compounds were characterized by either NMR pH titration, HPLC, or both. In this manner, all digestion products were assigned.

Gel mobility shifts were used to quantitate the extent of DNA bending [14]. Either a 9 or 12 pmol sample of DNA was boiled for 4 min and rapidly chilled on ice to remove secondary structure. To this DNA were added 3 μl of kinase/ligase buffer (700 mM, Tris, pH 7.6, 100 mM $MgCl_2$, and 50 mM dithiothreitol), 4 μl of ATP and 2 μl of polynucleotide kinase (New England Biolabs, 10000 U/ml). Unlabeled ATP (10 μM) was used to phosphorylate the platinated single strand or [γ-^{32}P]ATP (1.7 μM, spec. act. 5 mCi/ml) was added to radiolabel the complementary single-stranded DNA. The volume was adjusted to 30 μl. After allowing the reactions to proceed at 37 °C for 30 min, 1 μl of kinase/ligase buffer, 1 μl of 10 mM unlabeled ATP, 7 μl of H_2O, and 1 μl of T_4 polynucleotide kinase were added. After an additional 1 h at 37 °C, the phosphorylated oligomer was separated from unreacted ATP and T_4 polynucleotide kinase by using a Nensorb 20 nucleic acid purification cartridge (New England Nuclear).

9 pmol radiolabeled oligomer and 12 pmol unlabeled complement were added to 10 μl H_2O, brought to 90 °C, and allowed to cool slowly to room temperature in a water bath over a period of 6 h. The temperature was reduced to 4 °C for an additional 2 h. To the annealed oligomer (10 μl) were added 1 μl of 10 mM ATP, 7 mM H_2O, 2 μl of kinase/ligase buffer, and 1.5 μl of T_4 DNA ligase (Boehringer Mannheim, 9 U/ml). The ligation reaction was allowed to proceed overnight at 12 °C.

An aliquot of the ligation reaction mixture containing about 8000 cpm was loaded directly onto an 8% polyacrylamide gel (mono/bisacrylamide ratio = 29 : 1), 89 mM Tris-borate, 89 mM boric acid, and 2 mM EDTA. The gel slabs were mounted in a water-cooled electrophoresis apparatus and run at approx. 10 V/cm for 5 h at room temperature until the bromophenol blue dye marker had run the entire length of the 17 cm gel. The gels were fixed in 10% acetic acid, 10% methanol for 15 min and then dried with heat for 1 h followed by autoradiography for 15 h at −80 °C with a single intensifying screen.

The corresponding unplatinated oligomers were annealed, radiolabeled and ligated alongside their platinated counterparts and run as migration standards. In addition, *Bam*HI linker, d(CGGGATCCCG) (New England Biolabs), was radiolabeled and ligated as an additional migration standard according to the technical data sheet supplied. Migration distances of all bands on the gels were measured manually and by densitometry of the original autoradiogram.

3. Results

The sequences shown in fig. 1 were designed to ensure a unique orientation that guarantees fixed interplatinum distances in the polymers formed after ligation. All strands that were to be platinated were chosen to have guanosines and adenosines only where platination was desired. Since platinum reacts less well with cytosine and thymine, the sequence was completed with these two nucleotides in such a manner as to avoid runs of consecutive adenosines, which may contribute unwanted bending [6].

Platinum atomic absorption spectroscopy was used to determine the number of platinum atoms bound per oligomer. The amount of DNA present was estimated by using the extinction coefficient at 260 nm calculated for the unplatinated oligomer [22]. By this method we found that *cis*-GG contained 1.01 Pt/strand, *cis*-AG contained 1.08 Pt/strand, *cis*-GTG22 contained 1.27 Pt/strand, and *cis*-GTG15 contained 1.19 Pt/strand, while *trans*-GTG22 had 1.20 Pt/strand, *trans*-GTG15 had 1.20 Pt/strand, and (N3-C)-G had 1.09 Pt/strand. Since the ϵ_{260} values were not directly determined, and the effects of platination on the extinction coefficients were unaccounted for, the ratios are somewhat greater than unity.

Enzymatic digestion [19] was used to confirm the sequence content and identity of the platinum adduct in each modified oligonucleotide. The digestion products of all seven platinated single-stranded oligomers from fig. 1 were analyzed by reversed-phase HPLC at 260 nm as shown in fig. 2. The two major peaks in each chromatogram correspond to the excess of cytosine and thymine used to complete each platinated oligomer sequence. In each trace no guanosine or adenosine peaks are observable, confirming that these nucleotides reacted completely with platinum. The single remaining peak in each trace corresponds to the desired platinum adduct. Peaks arising from digestion of *cis*-GG, *cis*-AG, *cis*-GTG22, *cis*-GTG15, *trans*-GTG22, *trans*-GTG15, and (N3-C)-G correspond to the fragments *cis*-$[Pt(NH_3)_2\{d(GpG)\}]^+$, *cis*-$[Pt(NH_3)_2\{d(ApG)\}]^+$, *cis*-$[Pt(NH_3)_2(dG)_2]^{2+}$, *cis*-$[Pt(NH_3)_2(dG)]_2]^{2+}$, *trans*-$[Pt(NH_3)_2(dG)_2]^{2+}$, *trans*-$[Pt(NH_3)_2(dG)_2]^{2+}$, and *cis*-$[Pt(NH_3)_2(N3\text{-cytosine})(dG)]^{2+}$, respectively (fig. 2a–g). To confirm the identity of these peaks, and thus the nature of the adducts present in all platinated oligomers, model compounds (see above) were coinjected with the products of digestion of the platinated strands. In this manner, the platinum adducts present in each oligomer were determined to be unique and well defined.

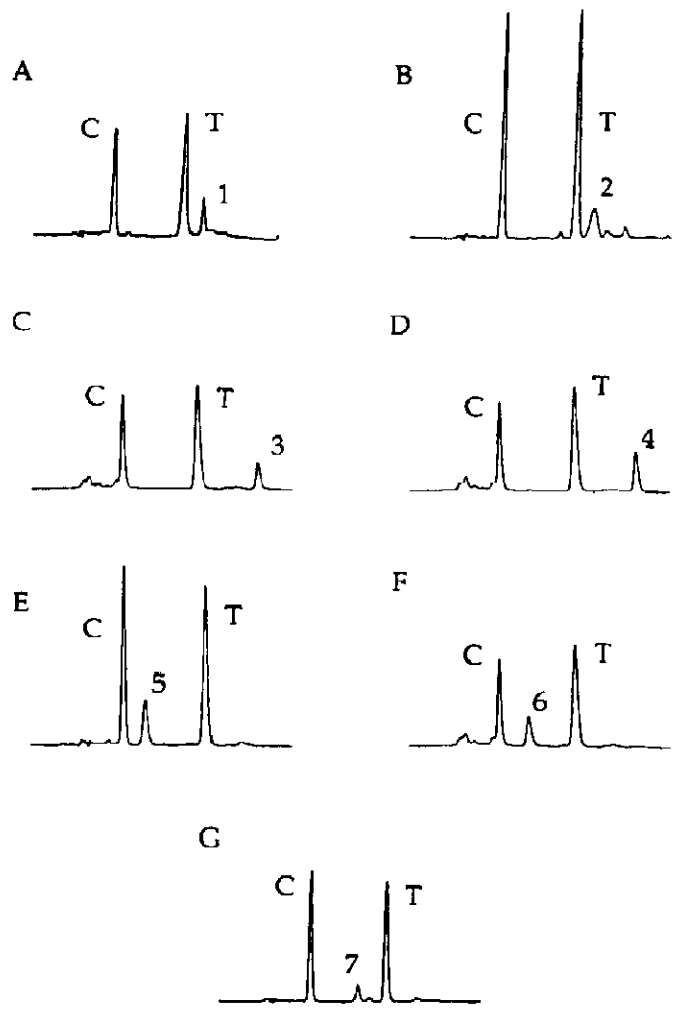


Fig. 2. Reversed-phase HPLC chromatograms (A_{260} vs. time) for the digestion products of the platinated single strands shown in fig. 1. (A) *cis*-GG, (B) *cis*-AG, (C) *cis*-GTG22, (D) *cis*-GTG15, (E) *trans*-GTG22, (F) *trans*-GTG15, and (G) (N3-C)-G. The two largest peaks in each trace correspond to cytosine and thymine, respectively. The third, smaller peak in each trace represents the platinum adduct incorporated into the original oligomer. Peaks were assigned by coinjection with model compounds as follows (1) *cis*-$[Pt(NH_3)_2\{d(GpG)\}]^+$, (2) *cis*-$[Pt(NH_3)_2\{d(ApG)\}]^+$, (3,4) *cis*-$[Pt(NH_3)_2(dG)_2]^{2+}$, (5,6) *trans*-$[Pt(NH_3)_2(dG)_2]^{2+}$, and (7) *cis*-$[Pt(NH_3)_2(N3\text{-cytosine})(dG)]^{2+}$.

The gel electrophoretic mobilities of the products of ligating the *cis*-GG, *cis*-AG and (N3-C)-G platinated 22 bp oligomers are shown in lanes 2, 3 and 5, respectively, of fig. 3. Lanes 1, 4 and 6 contain the unplatinated ligation products GG-STD, AG-STD and G-STD, respectively. As an

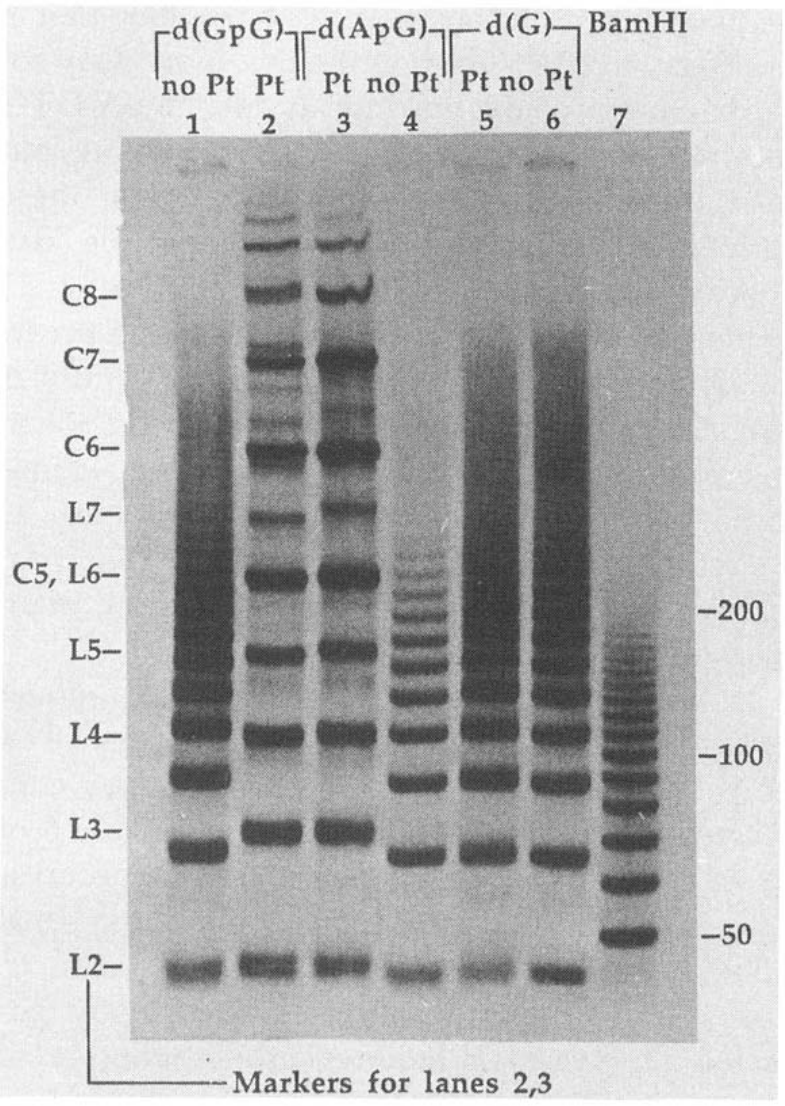


Fig. 3. Autoradiogram of products of ligation separated on an 8% native polyacrylamide gel. Lanes: (1) GG-STD, (2) *cis*-GG, (3) *cis*-AG, (4) AG-STD, (5) (N3-C)-G, (6) G-STD, and (7) *Bam*HI, where STD indicates the same oligomer shown in fig. 1, before platination.

additional molecular weight calibration standard, affording better resolution in the low molecular weight range, *Bam*HI linker was ligated and is included in lane 7. Calibration curves of the distance migrated vs. length in bp for the unplatinated oligomers were constructed in order to obtain the calculated length of a platinated oligomer based on its migration.

All three platinated oligomers in fig. 3 have nearly the same mobility as their unplatinated counterparts at the dimer level, where only two bends can add together. Platinated and unplatinated monomers were allowed to migrate off the bottom of the polyacrylamide gel. For *cis*-GG and *cis*-AG, migration differences between platinated and unplatinated oligomers, termed mobility shifts, become quite apparent in the longer polymers. In these longer molecules, many bends add constructively allowing the mobility shifts, and consequently the bend angles, to be quantitated with accuracy. The *cis*-AG oligomers show slightly greater mobility shifts than the *cis*-GG oligomers, indicating that the longer *cis*-AG polymers have shorter end-to-end distances than the corresponding *cis*-GG polymers. Also apparent in lanes 2 and 3 are a series of regularly spaced, darker bands, assigned as circular molecules. The smallest circle observed comigrates with the linear 6-mer. Such circular molecules were not observed in any of the unplatinated ligation reactions. The migration distances of these circular products were not analyzed quantitatively, but the formation of such large amounts of circular DNA clearly demonstrates the high degree of curvature caused by platinum binding which allows the ends of the DNA to approach each other and covalently close.

The (N3-C)-G multimers (shown in lane 5 of fig. 3) exhibit virtually no gel mobility shifts, migrating at almost exactly the same positions as the unplatinated ladder of multimers. In addition, no circular products were observed. These results show that DNA platinated with *cis*-[$Pt(NH_3)_2$-(*N*3-cytosine)Cl]Cl is not bent.

Fig. 4 displays the products of ligating the *cis* and *trans* 1,3 adducts. Lanes 4 and 6 contain multimers of *cis*-GTG with 22 and 15 bp sequence repeats, respectively. A 22 bp sequence repeat yields anomalously slowly migrating bands, and a population of circular DNA. The 22 bp phasing of platinum atoms corresponds approximately to two helical repeats, allowing bends to add constructively. The failure of the 15 bp oligomers to undergo gel shifts is due to the cancellation of bends when platinum is not phased properly, as previously reported [11].

The ligation products of *trans*-GTG22 and *trans*-GTG15 are depicted in lanes 3 and 5 of fig. 4. Lane 3 reveals a sequence of bands corresponding to linear multimers that are smeared and spaced at irregular intervals. These linear products appear to be split into two diverging series. Because of the peculiar nature of these bands, quantitation of the degree of bending due to the *trans*-GTG adduct was not attempted. Also present in

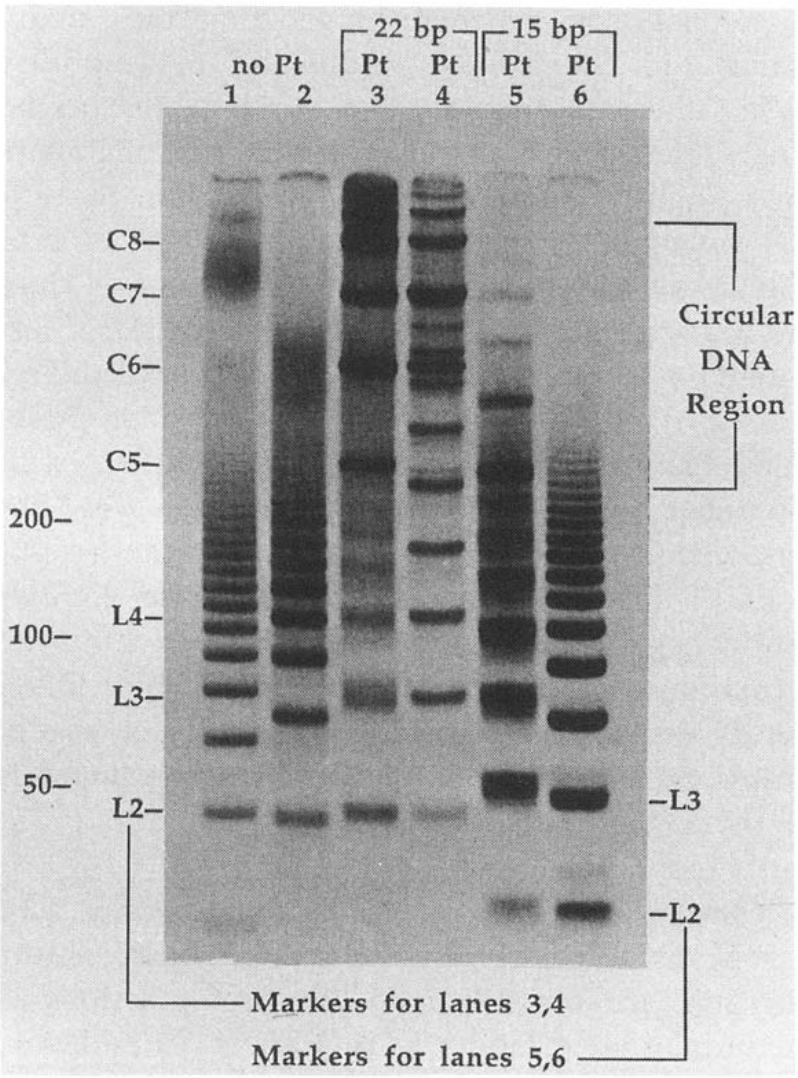


Fig. 4. Autoradiogram of the products of ligating *cis* 1,3 and *trans* 1,3 adducts. Lanes: (1) GTG15-STD, (2) GTG22-STD, (3) *trans*-GTG-22, (4) *cis*-GTG22, (5) *trans*-GTG15, and (6) *cis*-GTG 15.

lane 3 are a series of circular oligomers nearly comigrating with the circular products formed by the *cis*-GG and *cis*-AG oligomers. The presence of these circles indicates either increased flexibility or directed bending at the sites of platination [23,24]. Both phenomena would facilitate formation of covalently closed circles. To distinguish between the two possibilities, a 15 bp duplex DNA also containing the *trans*-GTG adduct was constructed. Because the platinum adducts are almost perfectly dephased in *trans*-GTG15, any directed bends will add destructively, preventing any appreciable anomalous mobility shifts or the formation of circles. Points of flexibility, or hinge joints, need not be positioned in phase to add constructively. Lane 5 contains ligation products of *trans*-GTG15. Clearly, the linear bands migrate more slowly than do their unplatinated couterparts of identical length. This result, and the fact that a series of circular DNA molecules is evident towards the top of the gel, indicate that *trans*-GTG does not bend the DNA in a rigid fashion, but rather increases its flexibility. The sizes of these circles are tentatively assigned by comparison with circles of known sizes [11] formed from ligating 22 bp platinated oligomers. The circle in lane 5 nearly comigrating with C5 (110 bp) in lanes 3 and 4 is assigned as a circular 7-mer (105 bp). Based on this assignment, the circular band in lane 5 comigrating with C7 (154 bp) in lanes 3 and 4 must be a circular 10-mer (150 bp). In this manner, all circular molecules in lane 5 can be assigned in an internally self-consistent fashion.

In order to determine whether the 1,3 adduct formed by cisplatin, *cis*-GTG, also renders DNA more flexible, the oligomer *cis*-GTG15 was constructed. Its ligation products appear in lane 6 of fig. 4. Evidently, no circular products are observable and the linear fragments have close to normal mobilities. Like the *cis*-GG and *cis*-AG adducts, *cis*-GTG also imparts a directed bend to the adducted DNA which requires proper phasing to add constructively.

The relative mobility (R_L) of a linear DNA fragment is defined as the ratio of calculated to actual length [6]. The calculated length is based only on a multimer's mobility, and is obtained from a calibration curve constructed from the mobilities of unplatinated linear DNA molecules of defined lengths. The greater the R_L value of an oligomer, the more slowly it moves compared to a straight molecule of the same length, and the greater its degree of curvature. The R_L values obtained for multimers of *cis*-GG, *cis*-AG, *cis*-GTG22, *cis*-GTG15, and (N3-C)-G of lengths 44 – 176 bp are plotted vs. length in bp in fig. 5. The greater the slope of a plot, the greater the degree of curvature of the DNA oligomer. Also plotted in fig. 5 for purposes of comparison are two curves based on A_6 tract induced bending [14]. The plot denoted A_6-1/2 contains one A_6 tract every two helix repeats and that labeled A_6-1/1 contains one A_6 tract every helix repeat. The slope of the plot for *cis*-AG is slightly greater than that for *cis*-GG, and the *cis*-GTG22 plot has a greater slope than that for either *cis*-GG or *cis*-AG. The

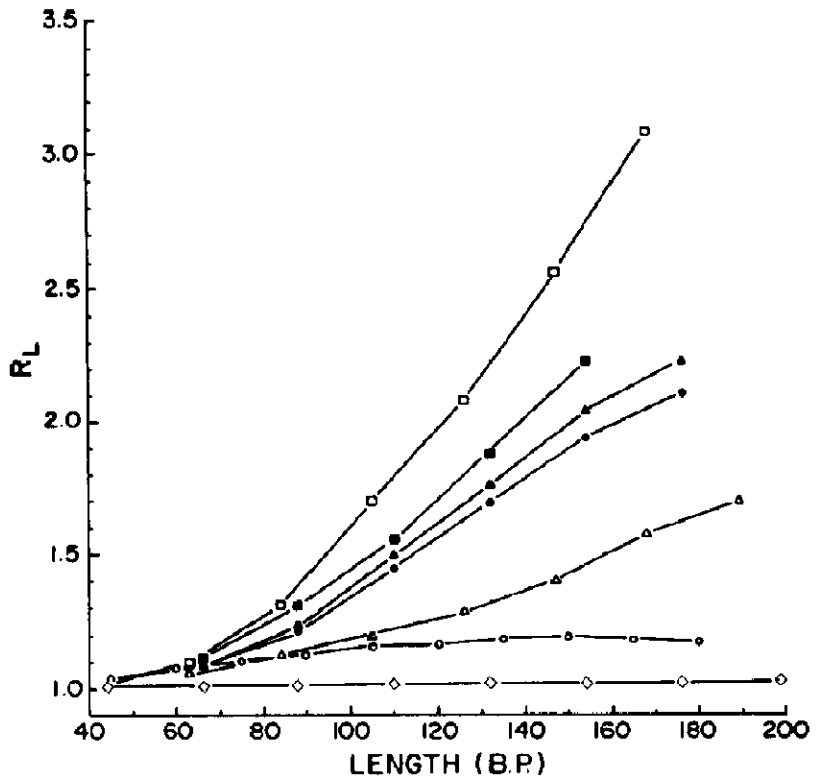


Fig. 5. Relative mobility (R_L) of all *cis* adducts (□) A_6-1/1, (■) *cis*-GTG22, (▲) *cis*-AG, (●) *cis*-GG, (△) A_6-1/2, (○) *cis*-GTG15, (◇) (N3-C)-G.

curves of all three platinated sequences fall between the A_6-1/1 and A_6-1/2 curves. Since *cis*-AG, *cis*-GG, *cis*-GTG22, and A_6-1/2 all contain one bend unit per 2 helix repeats, and A_6-1/1 has one bend per helix repeat, the magnitude of a platinum-induced bend is somewhere between those arising from one and two A tracts. The R_L vs. length curve for (N3-C)-G in fig. 5 appears as a horizontal line passing through the value R_L = 1.0, indicating no curvature. Also plotted in fig. 5 is the R_L vs. length curve for *cis*-GTG15. It rises slightly, then levels off, indicating an imperfectly phased bend unit [11,25].

Quantitation of the bend angles of *cis*-GG, *cis*-AG and *cis*-GTG is possible from the empirical relation given by eq. 1 [14],

$$R_L - 1 = (9.6 \times 10^{-5} L^2 - 0.47)(\mathrm{RC})^2 \qquad (1)$$

where RC represents the curvature relative to an A_6 tract and L the length of a particular oligomer with relative mobility R_L. This relation applies specifically to oligomers with perfectly phased bends having lengths between 120 and 170 bp. In such oligomers, where the sequence repeat exactly matches the helix repeat, the shape of the R_L vs. length curve is parabolic. Multimers of A_6-1/1 display this behavior quite nicely. For lengths greater than 140 bp, both *cis*-GG and *cis*-AG curves start to bow downward and deviate from a parabolic shape. This deviation can be due either to slightly improper phasing or to flexibility of the DNA helix. For these reasons we restrict our calculations to the 110 and 132 bp multimers, which lie on the parabolic portion of the R_L vs. length curve. Application of eq. 1 to the 110 and 132 bp fragments leads to curvatures of 0.80, 0.85, and 0.88 for *cis*-GG, *cis*-AG, and *cis*-GTG respectively, relative to an A_6 tract. The absolute degree of curvature in degrees is given by the relative curvature multiplied by the absolute value of an A_6 tract bend. Although this value is still subject to much debate, the most accurately determined value to date is 20° [26,27]. The results indicate that the bend due to the *cis*-GG adduct is about 32°, somewhat less than two A_6 tracts, and below the value of 40° previously reported [11]. The bend induced by the *cis*-AG adduct is greater by approx. 2°, namely, 34°, and that in the case of *cis*-GTG greater still, about 35°.

4. Discussion

Previous work on A tract phasing revealed that even single base-pair changes in the sequence repeat dramatically affect the shape of the resulting R_L vs. length curve [25]. Since bend angles are calculated from the shape of such a curve, the bends must be perfectly phased to compute an accurate angle using the gel electrophoretic shift method. In other words, the sequence repeat must match the natural helix repeat of the bent DNA. The sequence repeats of the *cis*-GG, *cis*-AG, *cis*-GTG, and *trans*-GTG platinated oligonucleotides were chosen to be 22 bp, or slightly greater than two helical turns of normal B-DNA, to reflect the fact that both *cis*- and *trans*-DDP unwind the double helix [28,29]. Since these unwinding studies were carried out on globally platinated supercoiled DNA, they do not necessarily reflect DNA unwinding due to the specific adducts *cis*-GG, *cis*-AG, *cis*-GTG or *trans*-GTG. Consequently, bends caused by different adducts cannot be rigorously compared unless they have been estab-

lished to be perfectly phased. The best example of such a case is the comparison between *cis*-GG and *cis*-AG bends, because the R_L vs. length curves for these two oligomers so closely parallel one another. Because the deviation from parabolic shape is approximately the same for both adducts, their phasing errors are probably similar, thereby enabling a rough comparison to be made between the two. While the calculated difference of 2° is experimentally reproducible, it is probably not significant compared to phasing errors. We therefore conclude that both *cis*-GG and *cis*-AG adducts bend the DNA helix by about 32–34°. Studies are currently in progress to address the effects of phasing in greater detail.

Both *cis*-AG and *cis*-GXG adducts have been previously reported to bend DNA [12], but the bend angles were not quantitated. These studies used a sequence repeat of 20 bp, which gave an R_L vs. length curve that deviated significantly from quadratic shape. The R_L vs. length curve for *cis*-GTG adducts with a 20 bp sequence repeat has a smaller slope than that for the *cis*-AG adduct [12]. Because the 22 bp sequence repeat used here gives R_L vs. length curves that more closely reflect parabolic behavior for both *cis*-AG and *cis*-GTG adducts, we conclude that the true helix repeat of *cis*-AG and *cis*-GTG adducted DNA is closer to 22 bp rather than 20 bp. At a 22 bp sequence repeat, the *cis*-GTG curve displays a greater slope. Comparison of these two adducts thus clearly illustrates the importance of proper phasing when estimating relative bend angles.

Inspection of the data produced from ligating the *trans*-GTG adduct reveals that bands due to linear DNA are smeared compared to linear bands formed in the three *cis*-DDP adducts studied. Bands for circular DNA from either *cis*- or *trans*-DDP-modified oligonucleotides are well delineated in all gels examined. In order to ensure that *trans*-GTG22 was intact, and that smearing was not due to a mixture of degradation products of the *trans*-DDP-platinated 22-mer, the single-stranded DNA was allowed to react with CN^- (0.3 M) to remove platinum [30]. The gel pattern obtained after polymerizing the cyanide-reversed oligomer matched perfectly that from ligation of the original unplatinated DNA. We therefore conclude that *trans*-GTG22 is intact, and that the anomalous gel electrophoresis results for ligation products of *trans*-GTG are not due to DNA degradation. Perhaps linear molecules with free ends are rendered more flexible by their *trans*-GTG adducts, sampling multiple conformations while running through the pores of the gel to produce the observed smearing. The circular molecules, restricted in conformational degrees of freedom because of their fixed ends, run as sharp bands like the *cis*-DDP adducted circles.

We have determined that polymers of *trans*-GTG adducted DNA yield DNA circles and anomalously slowly migrating linear molecules with a sequence repeat of either 2.0 or 1.5 helical repeats. These results establish the fact that the *trans*-GTG adduct can bend DNA in two directions perpendicular to the helix axis, phased approx. 180° apart. The present data do not allow one to demonstrate whether the two directions accessible to the DNA correspond to the major and minor grooves, or whether other directions of flexibility might be accessible. In order to address these points, one could explore various other sequence repeats, or vary the distance between *trans*-DDP adducts and a different, well-defined bending motif such as an A-tract [6,11].

From the present results, we conclude that no clear connection exists between the ability of a specific adduct to bend DNA and its inhibition of DNA replication. The *cis*-DDP adducts *cis*-GG, *cis*-AG and *cis*-GTG all impart directed bends to the double helix and block replication [3]. Although *trans*-DDP also blocks replication when bound to DNA [3], the adduct *trans*-GTG renders the double helix flexible, without affording a directed bend like the *cis*-DDP aducts. Most interestingly, the replication-blocking compound *cis*-$[Pt(NH_3)_2(N3\text{-cyt})Cl]Cl$ does not bend DNA at all [3].

Attempts have been made to correlate bending of various damaged DNA molecules with the rate of repair of the damage [7]. For example, thymine dimers, which bend DNA by 27–30° [23,31], are excised by the ABC excision nuclease [32] and by the repair enzyme *E. coli* DNA photolyase [33,34]. Interestingly, the ABC excision nuclease also excises damage from DNA globally platinated with

cis-DDP [35]. Studies on site-specifically platinated DNA indicate that the *cis*-GTG adduct is repaired faster by the ABC excision nuclease than the *cis*-GG or *cis*-AG adducts [36,37].

While the data available for DNA containing thymine dimers or modified by *cis*-DDP are consistent with the idea that bending could be a key structural motif that activates cellular repair machinery, results for psoralen-cross-linked DNA and *trans*-DDP-modified DNA do not support this hypothesis. It appears that psoralen cross-linking does not appreciably kink DNA [38], but the ABC excision nuclease system recognizes and removes site-specific psoralen damage from DNA [39,40]. *trans*-DDP-modified DNA is not a good substrate for the ABC excision nuclease complex [35]. Possibly, increased flexibility as a structural element cannot be recognized by the ABC excision nuclease, or perhaps the *trans*-GTG adduct characterized here is not representative of adducts formed upon global platination of DNA by *trans*-DDP. It would be interesting to test the recognition and repair of the specific *trans*-GTG and (N3-C)-G adducts by the ABC excision nuclease system in order to evaluate further this hypothesis.

Acknowledgements

This work was supported by Public Health Service Grant CA34992 from the National Cancer Institute. S.F.B. is grateful to the Whitaker Health Science Fund for a predoctoral fellowship. We also thank Professor D.M. Crothers and Dr. W.I. Sundquist for valuable discussions.

References

1 M.J. Cleare and J.D. Hoeschele, Bioinorg. Chem. 2 (1973) 187.
2 L.S. Hollis, A.R. Amundsen and E.W. Stern, J. Med. Chem. 32 (1989) 128.
3a A.L. Pinto and S.J. Lippard, Proc. Natl. Acad. Sci. U.S.A. 82 (1985) 4616; b: L.S. Hollis, W.I. Sundquist, J.N. Burstyn, W.J. Heiger-Bernays, S.F. Bellon, K.J. Ahmed, A.R. Amundsen, E.W. Stern and S.J. Lippard, manuscript in preparation; c: J.N. Burstyn, W.J. Heiger-Bernays and S.J. Lippard, manuscript in preparation.
4 R.B. Ciccarelli, M.J. Solomon, A. Varshavsky and S.J. Lippard, Biochemistry 24 (1985) 7533.
5 N. Sheibani, M.M. Jennerwein and A. Eastman, Biochemistry 28 (1989) 3120.
6 H.-M. Wu and D.M. Crothers, Nature 308 (1984) 509.
7 A. Sancar and G.B. Sancar, Annu. Rev. Biochem. 57 (1988) 29.
8 S.E. Sherman, D. Gibson, A.H.-J. Wang and S.J. Lippard, Science 230 (1985) 412.
9 G. Admiraal, J.L. van der Veer, R.A.G. de Graaff, J.H.J. den Hartog and J. Reedijk, J. Am. Chem. Soc. 109 (1987) 592.
10 a J. Kozelka, S. Archer, G.A. Petsko, S.J. Lippard and G.J. Quigley, Biopolymers 26 (1987) 1245.
10 b J. Kozelka, G.A. Petsko, S.J. Lippard and G.J. Quigley, J. Am. Chem. Soc. 107 (1985) 4079.
10 c J. Kozelka, G.A. Petsko, G.J. Quigley and S.J. Lippard, Inorg. Chem. 25 (1986) 1075.
11 J.A. Rice, D.M. Crothers, A.L. Pinto and S.J. Lippard, Proc. Natl. Acad. Sci. U.S.A. 85 (1988) 4158.
12 L. Marrot and M. Leng, Biochemistry 28 (1989) 1454.
13 D.P. Millar, K.M. Ho and M.J. Aroney, Biochemistry 27 (1988) 8599.
14 H.-S. Koo and D.M. Crothers, Proc. Natl. Acad. Sci. U.S.A. 85 (1988) 1763.
15 L.S. Lerman and H.L. Frisch, Biopolymers 21 (1982) 995.
16 O.J. Lumpkin and B.H. Zimm, Biopolymers 21 (1982) 2315.
17 J.C. Marini, S.D. Levene, D.M. Crothers and P.T. Englund, Proc. Natl. Acad. Sci. U.S.A. 79 (1982) 7664.
18 A.M.J. Fichtinger-Schepman, J.L. van der Veer, J.H.J. den Hartog, P.H.M. Lohman and J. Reedijk, Biochemistry 24 (1985) 707.
19 A. Eastman, Biochemistry 25 (1986) 3912.
20 A.M.J. Fichtinger-Schepman, A.T. van Oosterom, P.H.M. Lohman and F. Berends, Cancer Res. 47 (1987) 3000.
21 A. Eastman, M.M. Jennerwein and D.L. Nagel, Chem.-Biol. Interact. 67 (1988) 71.
22 G.D. Fasman, Handbook of biochemistry and molecular biology, 3rd edn., vol. 1, (1975) p. 589.
23 I. Husain, J. Griffith and A. Sancar, Proc. Natl. Acad. Sci. U.S.A. 85 (1988) 2558.
24 L. Ulanovsky, M. Bodner, E. Trifonov and M. Choder, Proc. Natl. Acad. Sci. U.S.A. 83 (1986) 862.
25 H.-S. Koo, H.-M. Wu and D.M. Crothers, Nature 320 (1986) 501.
26 S.D. Levene, H.-M. Wu and D.M. Crothers, Biochemistry 25 (1986) 3988.
27 H.-S. Koo, J. Drak, J.A. Rice and D.M. Crothers, Biochemistry, in the press.
28 G.L. Cohen, W.R. Bauer, J.K. Barton and S.J. Lippard, Science 203 (1979) 1014.
29 W.M. Scovell and L.R. Kroos, Biochem. Biophys. Res. Commun. 104 (1982) 1597.
30 S.J. Lippard and J.D. Hoeschele, Proc. Natl. Acad. Sci. USA 76 (1979) 6091.

31 D.A. Pearlman, S.R. Holbrook, D.H. Pirkle and S.-H. Kim, Science 227 (1985) 1304.
32 A. Sancar and W.D. Rupp, Cell 33 (1983) 249.
33 G.B. Sancar and A. Sancar, Trends Biol. Sci. 12 (1987) 259.
34 I. Husain and A. Sancar, Nucleic Acids Res. 15 (1987) 1109.
35 D.J. Beck, S. Popoff, A. Sancar and W.D. Rupp, Nucleic Acids Res. 13 (1985) 7395.
36 J.D. Page, I. Husain, A. Sancar and S. Chaney, Biochemistry 29 (1990) 1016.
37 J.D. Page, I. Husain, S.G. Chaney and A. Sancar, Platinum and other metal coordination compounds in cancer chemotherapy, Proceedings of the 5th Platinum Cancer Symposium, ed. M. Nicolini (Nijhoff, Boston, 1987) 115.
38 T.E. Haran and D.M. Crothers, Biochemistry 27 (1988) 6967.
39 B.V. Houten, H. Gamper, S.R. Holbrook, J.E. Hearst and A. Sancar, Proc. Natl. Acad. Sci. U.S.A. 83 (1986) 8077.
40 J.-M.H. Vos and P.C. Hanawalt, Cell 50 (1987) 789.