Sequence Specificity, Conformation, and Recognition by HMG1 Protein of Major DNA Interstrand Cross-links of Antitumor Dinuclear Platinum Complexes*

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Interactions of high mobility group (HMG) domain proteins with DNA modified by cisplatin plays a role in mechanisms underlying its antitumor activity. A structural motif recognized by HMG domain proteins on cisplatin-modified DNA is a stable, directional bend of the helix axis. In the present work, bending induced in DNA by major adducts of a novel class of antitumor compounds, represented by the formula \([(\text{trans-PtCl(NH}_3)_2\text{H}_2\text{N(CH}_2)_2\text{NH}_2\text{Cl})_2]\), was investigated. The oligodeoxyribonucleotide duplexes containing various site-specific interstrand cross-links of these bifunctional dinuclear platinum drugs were purified and characterized by Maxam-Gilbert footprinting, chemical probing, and phasing assay. It was demonstrated that the cross-links of the dinuclear compounds bent the helix much less than those of cisplatin. Gel retardation assay revealed very weak recognition of DNA adducts of dinuclear complexes by HMG1 protein. Hence, the mediation of antitumor properties of dinuclear platinum complexes by HMG domain proteins is unlikely so that polynuclear platinum compounds may represent a novel class of platinum anticancer drugs acting by a different mechanism than cisplatin and its analogues. A further understanding of how polynuclear platinum compounds modify DNA and how these modifications are processed in cells should provide a rational basis for the design of new platinum drugs rather than searching for cisplatin analogues.

Platinum coordination complexes are effective chemotherapeutic agents for the treatment of testicular cancer and are used in combination regiments for a variety of other tumors, including ovarian, cervical, bladder, lung, and those of the head and neck (1). The first platinum compound introduced in the clinic is cis-diaminedichloroplatinum(II) (cisplatin).1 Later, two new platinum complexes, cis-diammine-1,1-cyclobutanedi- carboxylatoplatinum(II) (carboplatin) and (trans-R, R)1,2-di-amminocyclohexanecarboxylatoplatinum(II) (oxaliplatin), were also introduced in the clinic, but they do not display a vastly different spectrum of antitumor activity (2, 3). The intrinsic along with acquired resistance and side effects observed in some patients represent major limitations of the treatment of human tumors with the platinum drugs currently used in the clinic. The need for improved clinical protocols has prompted a search for new platinum chemotherapeutic agents as well as a more complete understanding of the cellular mechanisms underlying both antitumor efficacy and resistance.

Platinum anticancer drugs exert their cytotoxic effect by inducing DNA damage with adducts formed being various types of cross-links (4–6). Since the discovery of the antitumor efficiency of cisplatin in 1969, the only new platinum antitumor drugs introduced in the clinic are direct structural analogues of cisplatin (cis-diammine-1,1-cyclobutanedicarboxylatoplatinum(II) differs from cisplatin only in the more inert leaving group similar to (trans-R, R)1,2-diaminocyclohexanecarboxylatoplatinum(II), which still contains as a carrier ligand 1,2-diaminocyclohexane). These new compounds produce an array of adducts very similar to those of cisplatin (7, 8). Therefore, it is not surprising that they induce similar biological consequences (2, 3, 9).

In a search for novel classes of platinum antitumor compounds a hypothesis that platinum drugs that bind to DNA in a fundamentally different manner to that of cisplatin will have altered pharmacological properties has been tested. This concept has already led to the synthesis of several new platinum antitumor compounds. One class of these novel compounds comprises bifunctional dinuclear and trinuclear platinum compounds that exhibit a different spectrum of cytostatic activity including activity in tumor cells resistant to cisplatin (10). It is therefore of great interest to understand details of molecular mechanisms underlying the biological efficacy of these new compounds, in particular how these compounds affect double-helical DNA.

Several recent papers have demonstrated unique DNA binding modes of these bifunctional polynuclear platinum compounds (11–16). We have shown (11, 14) that the compounds of general formula \([(\text{trans-PtCl(NH}_3)_2\text{H}_2\text{N(CH}_2)_2\text{NH}_2\text{Cl})_2\text{PtCl}_2\text{(trans-bisPt(n))}\] \(n = 2–6\); Fig. 1) preferentially form in DNA interstrand cross-links (~80%), and as a consequence of the global (random) modification of natural, high molecular mass DNA by these dinuclear platinum compounds, the conformation of this biomacromolecule is altered in a way that is dis-
RESULTS

Sequence Specificity of Interstrand Cross-linking—We demonstrated in our preceding papers (14) that the preferential DNA binding sites of trans-bisPt complexes are G residues and that the major DNA adducts of these dinuclear platinum compounds are 5'-guanine residues. The alkali labile bands were produced in the following manner. In the previous papers (17, 18) we investigated the formation of specific oligonucleotide adducts in the presence of trans-bisPt complexes (0.5 mM) by the addition of 0.5 mM AgNO₃ in 10 mM NaClO₄ at 37°C for 24 h in the dark. The AgCl precipitate was removed by centrifugation. The synthetic oligodeoxyribonucleotides (see Fig. 1B) were synthesized and purified as described previously (25). HMG1 proteins (for instance those containing high mobility group (HMG) domain; Refs. 20–22) were postulated to mediate the antitumor properties of the platinum drugs (23). Therefore, in addition to examining the structural alterations induced in DNA by the adducts of trans-bisPt compounds we also investigate in the present work how these adducts are recognized by HMG1 protein.

EXPERIMENTAL PROCEDURES

Chemicals—trans-bisPt(2), trans-bisPt(4), and trans-bisPt(6) (see Fig. 1A) were prepared as described previously (24). The mononucleotide species were generated from trans-bisPt complexes (0.5 mM) by the addition of 0.5 mM AgNO₃ in 10 mM NaClO₄ at 37°C for 24 h in the dark. The AgCl precipitate was removed by centrifugation. The synthetic oligodeoxyribonucleotides (see Fig. 1B) were synthesized and purified as described previously (25). HMG1 protein was a generous gift of Dr. M. Stros from the Institute of Biophysics (Brno, Czech Republic); it was isolated from calf thymus under nondenaturing conditions and purified and stored as described previously (26, 27). T4 DNA ligase and T4 polynucleotide kinase were purchased from New England Biolabs (Beverly, MA). Acrylamide, bis(acrylamide), urea, and NaCl were from Merck KGaA (Darmstadt, Germany). Dimethyl sulfate (DMS), KMnO₄, diethyl pyrocarbonate (DEPC), KBr, and KHSO₅ were from Sigma. [γ-³²P]ATP was from Amersham Pharmacia Biotech. Platinations of Oligonucleotides—The single-stranded oligonucleotides (24) were eluted from native polyacrylamide gels, precipitated by ethanol, and resuspended in 10 mM Tris-Cl (pH 7.4) with 1 mM EDTA buffer. The protein-DNA complexes were then resolved on a 7% polyacrylamide gel (29:1 acrylamide:N,N'-methylene-bis(acrylamide)). The samples were electrophoresed at 4°C using the electrophoresis buffer containing 0.045 M Tris borate and 1 mM EDTA, pH 8.0, and autoradiographed.

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In these formally bifunctional DNA binding dinuclear platinum agents, two monofunctional platinum(II) spheres with the single chloride leaving group on each platinum are linked by a variable length diamine chain so that the leaving chloride ligands are trans to the linker. Interestingly, varying the length of the linker may affect the distance and spatial configuration of the reactive chloride groups in these dinuclear compounds, hence also their DNA binding mode and consequently biological effect. Importantly, the observation that these dinuclear compounds preferentially form in DNA interstrand cross-links is also in marked contrast to cisplatin, which forms as major DNA adduct interstrand cross-links between neighboring purine residues, and the interstrand cross-links only represent minor DNA lesions (−6%; Refs. 17 and 18).

In the present work we continue to investigate DNA interactions of trans-bisPt complexes in cell-free medium to address further fundamental questions about the mechanism of antitumor activity of this novel class of platinum drugs. In a previous report (19), using an indirect assay employing exonuclease digestion of the DNA fragment globally modified only by one trans-bisPt complex (having n = 4), some sites in DNA involved in the interstrand cross-links have been already suggested. However, no systematic study on sequence specificity of these lesions including the effect of the length of the diamine linker has been performed. Therefore, first, we examined in detail which sites are preferentially involved in the major adducts of these dinuclear platinum compounds with particular attention paid to the effect of the length of their diamine linker. In addition, we also studied how major adducts of trans-bisPt compounds affect local conformation of DNA in particular bending and unwinding.

Some structures altered by platinum adducts, such as bending and unwinding attract various damaged DNA binding proteins (20) and contribute to the biological effect. Importantly, the observation that the top strands of all duplexes. The platinated top strands were allowed to react with T4 DNA ligase. The resulting samples along with ligated nonplatinated duplexes were subsequently examined on an Amersham Pharmacia Biotech FPLC System with MonoQ HR 5/5 column and a Unicam 939 AA spectrometer equipped with a graphite furnace, respectively. The platinations of Oligonucleotides—The modification by KMnO₄, DEPC, and KBr/KHSO₅ were performed as described previously (31–34). The strands of the duplexes were 5'-end-labeled with [γ-³²P]ATP. In the case of the platinated oligonucleotides, the platinum complex was removed after reaction of the DNA with the probe by incubation with 0.2 mM NaCN (alkaline pH) at 45°C for 10 h in the dark. Ligation and Electrophoresis of Oligonucleotides—Nonplatinated single-stranded oligonucleotides (Fig. 1B) and the duplexes containing a unique interstrand cross-link were 5'-end-labeled with [γ-³²P]ATP by using T4 polynucleotide kinase. Then the single-stranded, nonplatinated oligonucleotides were annealed with their phosphorylated complementary strands. Nonplatinated or cross-link containing duplexes were allowed to react with T4 DNA ligase. The resulting samples along with ligated nonplatinated duplexes were subsequently examined on 8% native polyacrylamide (mono:bis(acrylamide) ratio = 29:1) electrophoresis gels. Other details of these experiments were as described in previous published papers (35, 36). Some ligation products were also used as the DNA probes in the studies of the recognition of DNA adducts of trans-bisPt compounds by HMG1 proteins. For these experiments the double-stranded oligonucleotides 105 base pairs (bp) in length (containing five identical 21-bp oligonucleotide units d(TGTCT)/d(AGACA) (21)) were eluted from native polyacrylamide gels, precipitated by ethanol, and resuspended in 10 mM Tris-HCl/1 mM EDTA buffer, pH 7.4, to 5000 cpm/ml. For quantitation, the gel was stained with 0.5 mg of ethidium bromide/ml, and the amount of oligonucleotide duplex was determined in comparison with a known quantity of standard double-stranded oligonucleotide using a SepraScan 2001 image processing system for gel quantitation (ISS-Engprotec).

Gel Mobility Shift Assay—The study of HMG1-DNA binding complexes was carried out as described (37) with small modifications. 3 nM radiolabeled oligonucleotide probes 105 bp in length (concentration is related to the content of the 105-bp duplex), either nonmodified or containing the cross-link, were incubated for 15 min at 0°C (ice bath) in the presence of 50 or 100 nM HMG1 protein and 0.2 mg of nonlabeled, sonicated calf thymus DNA/ml in the binding buffer (150 mM NaCl, 10 mM MgCl₂, 20% glycerol, 0.2 mg of bovine serum albumin/ml, 10 mM HEPES-OH, pH 7.9, and 1 mM dithiothreitol) in a final volume of 10 μl. The protein-DNA complexes were then resolved on a 7% polyacrylamide gel (29:1 acrylamide/N,N'-methylene-bis(acrylamide)). The samples were electrophoresed at 4°C using the electrophoresis buffer containing 0.045 M Tris borate and 1 mM EDTA, pH 8.0, and autoradiographed.

RESULTS

Not all sequences were used in the experiments performed. The following is a list of the preferential DNA binding sites of trans-bisPt compounds that are G residues and that the major DNA adducts of these dinuclear platinum compounds are 5'-guanine residues.
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symmetrically to the single central cytosine (complementary to the platinated G in the top strand). In this way, the G residue in the top strand with the monofunctionally attached trans-bisPt complex could close to 1,2, 1,3, or 1,4 GG interstrand cross-links in the duplex 1,2, 1,3, or 1,4, respectively. The 2 interstrand cross-link is formed between G sites in neighboring base pairs, whereas in 1,3 and 1,4 interstrand cross-links, the platinated G sites are separated by one or two base pairs, respectively. G sites in the bottom strands involved in these interstrand cross-links are also in bold type in Fig. 1B. The nucleotide sequences of the duplexes were also designed in the way that these interstrand cross-links could close to G in the bottom strands located on both sides of the central C residue, i.e. in the 5’ → 5’ or 3’ → 3’ direction. The orientation of the interstrand cross-link in the 5’ → 5’ or 3’ → 3’ direction can be explained with the aid of the sequence of the duplex 1,3. For instance, the 1,3 GG interstrand cross-link oriented in the 5’ → 5’ direction is that formed in the duplex 1,3 between the central G in the top strand and G in position 10 in the bottom strand, whereas the same cross-link oriented in the 3’ → 3’ direction is that between the central G in the top strand and G in position 14 in the bottom strand.

The monoaducted top strands of the duplexes 1,2, 1,3, and 1,4 were hybridized with their complementary strands, and the hybrids were incubated in 0.1 M NaClO₄ at 37 °C. The aliquots were withdrawn at various time intervals and analyzed by gel electrophoresis under denaturing conditions. As shown in Fig. 2A for the duplex 1,2 modified by trans-bisPt(4), one band was only observed for the non-cross-linked duplex. The subsequent incubation resulted in new bands migrating markedly more slowly. Their intensity increased with the incubation time with a concomitant decrease in the intensity of the band corresponding to the non-cross-linked duplex. This observation can be interpreted to mean that interstrand cross-links were formed. From the ratio of the sum of intensities of all bands corresponding to cross-linked duplexes and the sum of intensities of all bands, the percentage of interstrand cross-links was calculated (Fig. 2B). The half-time of this interstrand cross-linking reaction in the duplex 1,3 was about 1 h. A similar result was obtained for the duplex 1,4, whereas the half-time found for the duplex 1,2 was markedly higher. The kinetics of the interstrand cross-linking reactions in all three duplexes were approximately the same and independent of the linker length of the trans-bisPt complex (n = 2, 4, or 6) (not shown).

The cross-linking reactions in the duplexes 1,2, 1,3, and 1,4 resulted in only one band or single peak in the FPLC profile designated as inter in Fig. 2A (shown for the duplex 1,2 and trans-bisPt(4)). After a 24-h reaction period, the bands or FPLC peaks corresponding to the interstrand cross-linked duplexes were cut off from the gel or collected after FPLC separation, respectively, and the duplexes were isolated (eluted from the bands or precipitated or only precipitated from the FPLC fractions) and further characterized by Maxam-Gilbert footprinting (28, 31, 38).

The samples of the 1,2, 1,3, and 1,4 interstrand duplexes cross-linked by either trans-bisPt(2, 4, 6) in which the upper strand was only 5’-end labeled with 32P were reacted with DMS, which does not react with platinated G because the N-7 position is no longer accessible (28, 31, 38). The adducts were removed by NaCN (28, 39), and then the sample was treated with piperidine. In the nonplatinated duplexes, the central G in the top strands was reactive with DMS (not shown). It was no longer reactive in all three cross-linked duplexes. This observation confirms that the unique G in the upper strands remained platinated and was involved in the interstrand cross-link contained in the single fraction of interstrand cross-linked complexes are interstrand cross-links. In these cross-links the platinated sites are separated by one or more base pairs. Bi-functional dinuclear platinum complexes must bind to DNA first through one end of the dinuclear unit. In this first step of binding, the kinetic preferences are similar to those of mononuclear species, i.e. they coordinate preferentially to N-7 atoms of G residues. The array of adducts becomes, however, different upon the coordination of the second platinum unit (19). Considering these facts we have designed a series of synthetic oligodeoxyribonucleotide duplexes 1,2, 1,3, and 1,4, whose sequences are shown in Fig. 1B. The pyrimidine-rich top strands of these duplexes only contained one G in the central G site. The duplexes were also designed in such a way that their bottom (complementary) strands contained G in different positions

Fig. 1. Structures of dinuclear platinum complexes (A) and sequences of the synthetic oligodeoxyribonucleotides used in the present study with their abbreviations (B). The top and bottom strands of each pair are designated top and bottom, respectively, in the text. The bold letter in the top strands of all duplexes except d(TGGT)/d(ACCCG) indicates the location of the monofunctional adduct of trans-bisPt complexes formed before interstrand cross-linking reaction in the way also described in the experimental section. The bold letters in the top strand of d(TGGT)/d(AGCCA) duplex indicate the location of the intrastrand cross-link after modification of the oligonucleotides by trans-bisPt complexes or cisplatin in the way described under “Experimental Procedures.” For the duplex 1,3, the numbering of the nucleotide residues in the bottom strand is also shown.
Fig. 2. Kinetics of DNA interstrand cross-link formation by trans-bisPt(4) complex. The duplexes 1,2, 1,3, and 1,4 were formed by mixing their bottom strand with the complementary upper strand uniquely monoadducted by trans-bisPt(4) at the central G residue at 37 °C. A, autoradiogram of a 12% polyacrylamide/8 M urea denaturing gel of the duplex 1,2 whose bottom strand was 32P end-labeled. The cross-linking reaction was stopped by adjusting the NaOH concentration to 10 mM and cooling the samples to −70 °C. inter designates bands corresponding to the interstrand cross-linked fraction (see also the text). Lane 1, 0 h; lane 2, 2 h; lane 3, 4 h; lane 4, 17.5 h; lane 5, 24 h; lane 6, 48 h. B, the percentage of interstrand cross-linking in the duplexes 1,2 (●), 1,3 (▲), and 1,4 (▼) by trans-bisPt(4) calculated from the ratio of the sum of the intensities of the bands corresponding to the fragments containing an interstrand cross-link to the sum of the intensities of all bands (corresponding to the non-cross-linked and the cross-linked oligonucleotides).

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duplexes (28, 31, 38).

In further studies the 1,2, 1,3, and 1,4 interstrand duplexes in which the bottom strand was 5′-end labeled with 32P were examined. Results of typical experiments, in which the locations of nucleotides involved in the interstrand cross-link of trans-bisPt(4) formed in the duplexes 1,2, 1,3, or 1,4 were determined, are shown in Fig. 3. The interstrand cross-linked duplexes were reacted with DMS. Then these samples were further treated with NaCN to remove the adducts and finally also with piperidine. The treatment with piperidine of the control, nonplatinated duplex resulted in the cleavage at all G sites in the bottom strand (Fig. 3, no Pt lanes). If the cross-linked duplexes treated with DMS and subsequently NaCN were cleaved (Fig. 3, Pt/NaCN lanes), the bands corresponding to all G residues were observed except G residues marked by an arrow in Fig. 3. This result proves that these G residues were platinated and involved in the interstrand cross-link of the trans-bisPt(4) compound. Identical results of these footprinting experiments were obtained if trans-bisPt(2) or trans-bisPt(6) were used to cross-link the duplexes 1,2, 1,3, or 1,4. It implies that the interstrand adducts formed by the trans-bisPt complex were GG interstrand adducts formed at the G site in the bottom strand exclusively in the direction to its 5′ end (oriented in the 5′ → 5′ direction) and that the sites of interstrand cross-linking by trans-bisPt complexes were not substantially affected by the length of the linker chain.

Chemical Probes of DNA Conformation—Because 1,2 interstrand cross-links represent only less frequent lesions formed in DNA by trans-bisPt complexes, further studies of the present work were focused mainly on the major 1,3 interstrand cross-link. The oligonucleotide duplexes containing single 1,3 interstrand cross-link between G residues oriented in the 5′ → 5′ direction were further analyzed by chemical probes of DNA conformation. The interstrand cross-linked duplexes were treated with several chemical agents that are used as tools for monitoring the existence of conformations other than canonical B-DNA. These agents include KMnO4, DEPC, and bromine. They react preferentially with single-stranded DNA and distorted double-stranded DNA (31–34, 40). In the following text the analysis of the duplex d(TGTCT)/d(AGACA) (21) (see Fig. 1B for its sequence) containing 1,3 interstrand cross-link of trans-bisPt(4) is demonstrated as a general example.

KMnO4 is hyperreactive with thymine residues in single-stranded nucleic acids and in distorted DNA as compared with B-DNA (32, 34, 41, 42). KMnO4 reacted with no residue within the nonplatinated duplex (Fig. 4A, lane ss). All T residues were strongly reactive in the nonplatinated single-stranded top oligonucleotide (Fig. 4A, lane ss). The interstrand cross-linked duplex showed strong reactivity of the 5′ T residue adjacent to the adduct (Fig. 4A, lane dsPt). A somewhat weaker reactivity was also observed for the second 5′ T and the 3′ T adjacent to the platinated G involved in the cross-link.

DEPC carbethoxylates purines at the N-7 position. It is hyperreactive with unpaired and distorted adenine residues in DNA and with left-handed Z-DNA (32, 34, 43, 44). A and G residues within the nonplatinated single-stranded oligonucleotide (top and bottom) readily reacted with DEPC (shown for the bottom strand in Fig. 4B, lane ds). Within the double-stranded oligonucleotide containing the interstrand cross-link, three base residues in the bottom strand became reactive (Fig. 4B, lane dsPt). These are readily identified as the three A residues complementary to the reactive T residues of the top strand. Importantly, A residues complementary to strongly reactive T residues also reacted with DEPC strongly, whereas those A residues complementary to more weakly reactive T residues also reacted with DEPC, only more weakly.
Bromination of C residues and formation of piperidine-labile sites are observed when two simple salts, KBr and KHSO₃, are allowed to react with single-stranded or distorted double-stranded oligonucleotides (33). The reaction proceeds via generation of Br₂, in situ, which reacts selectively with the 5,6 double bond to add Br and OH, respectively. H₂O is then eliminated to give 5-bromodeoxyuridine, which is susceptible to depyrimidination under basic conditions. All C residues within the nonplatinated single-stranded top or bottom strands of the d(TTGTCT)/d(AGACAA) duplex were strongly reactive (Fig. 4, C and D, lanes ss). No reactivity of these residues was observed within the nonplatinated duplex (Fig. 4, C and D, lanes ds). Within the double-stranded duplex containing the cross-link no C residue in the top strand, including that complementary to the G residue in the bottom strand involved in the cross-link was reactive (Fig. 4C, lane dsPt). In contrast, the only C residue in the bottom strand (complementary to the platinated G residue in the top strand) was strongly reactive (Fig. 4D, lane dsPt).

The results of the analysis of the d(TTGTCT)/d(AGACAA) (21) duplex containing 1.3 GG interstrand cross-link of trans-bisPt(4) by chemical probes are summarized in Fig. 4E. Importantly, identical results as demonstrated in Fig. 4 were obtained also for the duplex containing the cross-link of trans-bisPt(2) and (6) compounds.

DNA Unwinding and Bending—Among the alterations of secondary and tertiary structure of DNA to which it may be subject, the role of intrinsic bending and unwinding of DNA is increasingly recognized as being of potential importance in regulating replication and transcription functions through specific DNA-protein interactions. For DNA adducts of cisplatin, the structural details responsible for bending and subsequent protein recognition have recently been elucidated (21, 23). Given the recent advances in our understanding of the structural basis for the bending of DNA caused by cisplatin cross-links, it is of considerable interest to examine how major DNA adducts of trans-bisPt compounds (interstrand cross-links) affect conformational properties of DNA such as bending and unwinding. In this work we further performed studies on the bending and unwinding induced by single, site-specific 1,3 GG interstrand cross-link of trans-bisPt(4) formed in the oligodeoxyribonucleotide duplexes using electrophoretic retardation as a quantitative measure of the extent of planar curvature.

The oligodeoxyribonucleotide duplexes d(TTGTCT)/d(AGACAA) (20–23) (20–23 bp, for their sequence see Fig. 1B) were used for the bending and unwinding studies of the present work. All sequences were designed to leave a 1-nucleotide overhang at their 5′-ends in double-stranded form. These overhangs facilitate polymerization of the monomeric oligonucleotide duplexes by T4 DNA ligase in only one orientation and maintain a constant interadduct distance throughout the resulting multimer. Autoradiograms of electrophoresis gels revealing resolution of the ligation products of nonplatinated 20–23-bp duplexes or containing a unique 1,3 GG interstrand cross-link of trans-bisPt(4) are shown in Fig. 5. A small but significant retardation was observed for the multimers of all platinated duplexes. Decreased gel electrophoretic mobility may result from a decrease in the DNA end-to-end distance (45). Various platinum(II) complexes have been shown to form DNA adducts that decrease gel mobility of DNA fragments because of either stable curvature of the helix axis or increased isotropic flexibility (31, 36, 46–48). DNA multimers of identical length and number of stable bend units, but with differently phased bends, have different end-to-end distances. The DNA bends of a multimer must therefore be spaced evenly and phased with the DNA helical repeat to add constructively. Such constructively phased bends add in plane, yielding short end-to-end distances and the most retarded gel migration. In other words, gel electrophoresis of multimers of oligonucleotide duplexes that only differ in length and contain a stable curvature induced by the same platinum adduct should exhibit a phase effect, i.e. the maximum retardation should be observed for the multimers having the bends in phase with helix screw. In contrast, the normal electrophoretic mobility should be observed for the multimers having the bends separated by a half-integral number of DNA turns. The K factor is defined as the ratio of calculated to actual length. The calculated length is based on a multimer’s mobility and is obtained from a calibration curve constructed from the mobilities of nonplatinated multimers. The variation of the K factor versus sequence length obtained for multimers of the duplexes 20–23 bp long and containing the unique 1,3 GG interstrand cross-link of trans-bisPt(4) is shown in Fig. 6A. Maximum retardation was observed for the 21-bp cross-linked duplex. The 20- and 22-bp curves exhibited smaller but different slopes. This observation suggests that the natural 10.5-bp repeat of B-DNA and that of DNA perturbed by the interstrand cross-link of trans-bisPt(4) are slightly different as a consequence of DNA unwinding (49). In other words, this asymmetry is consistent with DNA unwinding due the formation of the interstrand cross-link of trans-bisPt(4) compound.

The exact helical repeat of the interstrand cross-linked duplex and from it the unwinding angle were calculated by inter-
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Fig. 4. Chemical probes of DNA conformation. Piperidine-induced specific strand cleavage at KMnO₄-modified (A), DEPC-modified (B), and KBr/KHSO₅-modified (C and D) bases in the duplex d(TGTCT)/d(AGACA) (21) nonplatinated or containing single 1,3 interstrand cross-link of trans-bisPt(4). The oligomers were 5’-end labeled at their top or bottom strands. A, KMnO₄, only top strand end labeled. Lane ss, the nonplatinated top strand; lane ds, the nonplatinated duplex; lane dsPt, the duplex interstrand cross-linked by trans-bisPt(4); lane G, a Maxam-Gilbert specific reaction for the nonplatinated duplex. B, DEPC, only bottom strand end labeled. Lane ss, the nonplatinated top strand; lane ds, the nonplatinated duplex; lane dsPt, the duplex interstrand cross-linked by trans-bisPt(4); lane C+T, a Maxam-Gilbert specific reaction for the nonplatinated duplex. C, KBr/KHSO₅, only top strand end labeled. Lane ss, the nonplatinated top strand; lane ds, the nonplatinated duplex; lane dsPt, the duplex interstrand cross-linked by trans-bisPt(4); lane G, a Maxam-Gilbert specific reaction for the nonplatinated duplex. D, KBr/KHSO₅, only bottom strand end labeled. Lane ss, the nonplatinated top strand; lane ds, the nonplatinated duplex; lane dsPt, the duplex interstrand cross-linked by trans-bisPt(4); lane C+T and G, Maxam-Gilbert specific reactions for the nonplatinated duplex. E, summary of the reactivity of chemical probes; ○, 9, and ○ designate strong, medium and weak reactivity, respectively.

The evaluation of the relationship between interadduct distance and phasing for self-ligated multimers composed of the identical number of monomeric duplexes (bend units) resulted in a bell-shaped pattern (Fig. 6B) characteristic for bending (31, 36, 46–48, 52). The quantitation of the bend angle of the 1,3 interstrand cross-link of trans-bisPt(4) was performed in the way described previously (31, 36, 46–48, 52) utilizing the following empirical equation.

\[ K - 1 = (9.6 \times 10^{-3} L^2 - 0.47)(RC)^2 \]

(Eq. 1)

where \( L \) represents the length of a particular oligomer with relative mobility \( K \), and \( RC \) represents the curvature relative to a DNA bending induced at the tract of A residues (A tract) (46, 53). Application of Equation 1 to the 105-, 126-, or 168-bp multimers of the 21-bp oligomer containing the single 1,3 interstrand cross-link of trans-bisPt(4) leads to a mean curvature of 0.25, relative to the A tract. The average bend angle per helix turn can be calculated by multiplying the relative curvature by the absolute value of the A tract bend (20°; Refs. 36, 46, 53, and 54). The results indicate that the bend induced by the 1,3 GG interstrand cross-link of trans-bisPt(4) is only about 10°. Other details of the calculations of the unwinding and bending angles are given in the previously published papers (31, 36, 46–48, 52). Importantly, similarly small unwinding and bending angles were yielded by the analysis of 1,3 GG interstrand cross-links formed by other two trans-bisPt compounds (\( n = 2 \) and 6) (not shown). Also importantly, small values of bending and unwinding angles similar to those deduced from the experiments shown in Figs. 5 and 6 were produced by the 1,4 GG interstrand cross-links formed by all three trans-bisPt(2, 4 or 6) compounds as well.

Recognition by HMG1 Protein—The bending of the helix axis induced by DNA intrastrand and interstrand cross-links of cisplatin and the altered structure attract HMG and other proteins (20, 22). This binding of HMG domain proteins to cisplatin-modified DNA has been postulated to mediate the antitumor properties of this drug (21, 23). Because bifunctional trans-bisPt and other dinuclear compounds exhibit antitumor activity different from cisplatin, it was of considerable interest to examine how the adducts of trans-bisPt compounds are recognized by HMG domain proteins. The interactions of the HMG1 protein, which is the prototypical member of a family of these proteins, with major DNA intrastrand cross-links of trans-bisPt compounds were investigated by means of gel mobility shift experiments (Fig. 7). In these experiments, the duplex d(TGTCT)/d(AGACA) (21) was modified so that it contained a single, defined 1,3 GG interstrand adduct. These duplexes were radioabeled and ligated to multimers. After the ligation reaction, the DNA probes composed of five duplex oligonucleotide units were purified. Thus, these 105-bp DNA probes contained five identical interstrand cross-links regularly separated by identical 21-bp segments. Similar DNA probes of such length were already used in the previous work (22, 55) to demonstrate a specific binding of the DNA damage recognition proteins to DNA adducts of cisplatin. These proteins, identified as the HMG domain proteins (56), were found to bind the probes similar to those used in this work, which were at least 88 or more bp long (55).

The binding of the HMG1 protein to these DNA probes in the presence of 2,000-fold excess of nonlabeled nonspecific calf thymus competitor DNA was detected by retardation of the migra-
tion of the radiolabeled 105-bp probe through the gel (22, 55, 56) (Fig. 7). There is a specific binding of the HMG1 protein to the DNA probe containing the 1,3 GG interstrand cross-link of trans-bisPt(4) as evidenced by the presence of a more slowly migrating band that is not seen for the same duplex analyzed in the absence of the HMG1 protein (Fig. 7, lanes 6–8). To compare the binding affinity of the HMG1 protein to the 1,3 GG interstrand cross-link of trans-bisPt compounds and to the 1,2-(GpG) intrastrand cross-link of cisplatin, a DNA probe was also prepared so that it contained this cisplatin adduct. This probe was prepared by ligation of five 21-bp oligonucleotide units (d(TGGT)/d(ACCA); see Fig. 1B for its sequence), each containing the single central sequence d(GpG)/d(CpC) at which the intrastrand cross-link of cisplatin was formed so that this DNA probe was also 105 bp in length. As expected, this radiolabeled intrastrand cross-linked probe readily bound the HMG1 protein in the presence of 2,000-fold excess of nonlabeled nonspecific calf thymus competitor DNA (Fig. 7, lane 4).

The densitometric evaluation of the bands in Fig. 7 showed that the affinity of HMG1 protein to the 1,3 GG interstrand cross-link of trans-bisPt(4) was approximately 1 order of magnitude lower. Again, identical results were obtained in the experiments using the same DNA probe, but containing the 1,3 interstrand cross-link of trans-bisPt(2 and 6) or 1,4 interstrand cross-link of all three trans-bisPt compounds tested in the present work (not shown). Also importantly, no binding of the protein occurred under identical experimental conditions in the cases where the same 105-bp DNA probes were not platinated (Fig. 7, lane 2).

trans-bisPt compounds may also form as a minor DNA adduct intrastrand cross-link between two neighboring G residues (1,2 (GpG) intrastrand cross-link), which is the direct analogue of the major cisplatin adduct (19). Recently, we described a structural analysis of this intrastrand cross-link of trans-bisPt compounds (12). The formation of this adduct resulted in conformational alterations distinctly different from those produced by the same adduct of cisplatin. One of the major differences comprised a flexible nondirectional bend in contrast to the same adduct of cisplatin, which produces in DNA a stable directional curvature (bends helix axis toward major groove by 30–60°; Refs. 36 and 57–59). It has been predicted on the basis of this result (12) that this intrastrand cross-link of trans-bisPt complex should be recognized by HMG domain proteins markedly less efficiently than the same cross-link formed in DNA by cisplatin. To determine how the 1,2-DNA Modifications by Dinuclear Platinum Compounds
cisplatin.

lower than that of intrastrand or interstrand cross-links of HMG1 protein or that their affinity to this protein is markedly trans strand adducts of bisPt(2 or 6) (not shown). From these results and the results HMG1 protein used in these experiments. The identical results indicated in Fig. 7 (lanes 10–12). No more slowly migrating band indicating the specific binding of the HMG1 protein to the DNA probe containing 1,2-(GpG) intrastrand cross-links of trans-bisPt(4) to HMG1 protein are illustrated in Fig. 7 (lanes 10–12). No more slowly migrating band indicating the specific binding of the HMG1 protein to the DNA probe containing 1,2-(GpG) intrastrand cross-links of trans-bisPt(4) to HMG1 protein is noticed even at the highest concentrations of HMG1 protein used in these experiments. The identical results were obtained with the probe containing these adducts of trans-bisPt(2 or 6) (not shown). From these results and the results demonstrating the affinity of HMG1 protein to the major adduct of trans-bisPt compounds, it is clear that the DNA intrastrand adducts of trans-bisPt compounds are not recognized by HMG1 protein or that their affinity to this protein is markedly lower than that of intrastrand or interstrand cross-links of cisplatin.

**DISCUSSION**

The sequence specificity of interstrand cross-link formation in DNA by trans-bisPt compounds has been assayed in the present work by Maxam-Gilbert footprinting. The experiments presented here (Figs. 2B and 3) clearly demonstrate that preferential DNA binding sites in these lesions are G residues in the base pairs separated by at least one another base pair. The cross-links between G residues in neighboring base pairs (1,2 interstrand cross-links) are formed with a pronouncedly slower rate (Fig. 2B) so that it is reasonable to suggest that 1,2 GG interstrand cross-links represent less frequent adducts of trans-bisPt compounds. Thus, the formation of long range cross-links by trans-bisPt compounds proposed in earlier papers (14, 19) and hence the ability of these dinuclear platinum compounds to target larger sequences of DNA has been confirmed in this study. Our analysis has also demonstrated that the sites involved in DNA interstrand cross-linking by trans-bisPt compounds are essentially identical for \( n = 2, 4, \) and \( 6 \) so that the length of the diamine bridge linking the two platinum units does not appear to be a substantial factor affecting DNA interstrand cross-linking by these dinuclear platinum compounds. Importantly, the results of the present work (Fig. 3) have also confirmed under competitive conditions that the interstrand cross-links of trans-bisPt complexes are preferentially formed in the 5′ → 5′ direction. The reasons for this preference in the orientation of DNA interstrand cross-links of trans-bisPt compounds are unknown. Interestingly, the same preference in the orientation of interstrand cross-links is also observed for the geometric isomers of trans-bisPt compounds, \([\text{cis-PtCl(NH}_3)_2\text{H}_2\text{N(CH}_2)_n\text{NH}_2\text{Cl}]_2, n = 4, 6, 8, 12\) (which have left chloride ligands cis to the linker) (15) so that this feature of the interstrand cross-linking might be common for this class of platinum compounds.

The present paper also describes the conformational distortion induced in DNA by the 1,3 GG interstrand cross-links of trans-bisPt complexes. The bending experiments were carried out with the double-stranded oligodeoxyribonucleotides d(TGTCT)/d(AGACA) (20–23) (Fig. 1B) containing the unique interstrand cross-link in their central sequence. The phasing assay (Figs. 5 and 6) has revealed that the 1,3 GG interstrand cross-link of trans-bisPt complexes results only in a very small directional bending of helix axis (−10°) and duplex unwinding (−9°), and basically the same result was obtained for the 1,4 GG interstrand cross-link.

Despite these small bending and unwinding effects the major interstrand cross-links formed by trans-bisPt compounds in the d(TGTCT)/d(AGACA) (21) duplex created a local conformational distortion revealed by the chemical probes (Fig. 4). This distortion was nonsymmetrical and extended mainly over four base pairs (Fig. 4E). A careful examination of these results reveals that mainly base pairs containing pyrimidine bases, two 5′ and one 3′ to the platinated G in the top strand were distorted. This observation may reflect the fact that the long range cross-links of bifunctional dinuclear platinum compounds affect DNA in the first approximation almost as two independent monofunctional adducts of mononuclear platinum(II) complexes. It has been shown (25, 60) that monofunctional adducts of mononuclear platinum(II) complexes also considerably distort DNA, but in a sequence-dependent manner. Major extensive distortions in base pairs localized around the monofunctional adduct of mononuclear platinum(II) complexes at G residue were observed only if such a platinated G was flanked by pyrimidine residues. In contrast, no or weak distortions were noticed using chemical probes if the platinated G was flanked by purine residues particularly on its 5′ site. Thus, if the G residues involved in the 1,3 interstrand cross-link of trans-bisPt complex in the d(TGTCT)/d(AGACA) duplex are substituted by the same G residues at which monofunctional adduct of mononuclear platinum(II) complex was formed, a reactivity pattern of chemical probes similar or identical to that seen in Fig. 4E is obtained. In other words, the platinated G residue in the top strand (involved in the interstrand cross-
link) is flanked by pyrimidine residues, and hence the base pairs adjacent to this G residue are distorted. In contrast, the second platinated G in the bottom strand (involved in the cross-link) is flanked by purine residues so that in accord with what has been deduced above the base pairs adjacent to this second platinated G are not distorted or are distorted much less extensively. That long range cross-links of trans-bis-Pt complexes distort duplex similarly as two close but independent monofunctional adducts of mononuclear platinum(II) complexes is also sustained by the observation that these long range interstrand cross-links unwind DNA by only ~9°, which is a value close to the unwinding angle produced by monofunctional adducts of mononuclear platinum(II) complexes (~6°; Ref. 61). Similarly, no bending of helix axis by these monofunctional platinum(II) adducts was observed (25), which is again close to the small values of ~10° found for 1,3 and 1,4 interstrand cross-links of trans-bis-Pt compounds in the present work.

Thus the results of the present work indicate that some localized conformational features of major DNA adducts of antitumor trans-bis-Pt compounds are similar to those of monofunctional mononuclear platinum(II) compounds, such as chlorodiethylenetriamineplatinum(II) chloride (PtCl(dien))Cl or [PtCl(NH₃)₃]Cl, which are clinically ineffective. The chemical probes, which revealed these similarities, allow detecting the sites where the distortion is localized and its extent, but they do not provide all details about the character or nature of the distortion. It has been shown (11, 14, 62) with the aid of various methods of molecular biophysics, such as for instance circular dichroism of DNA, DNA melting curves, and immunochemical analysis, that on the other hand several features of conformational distortions induced in DNA by dinuclear platinum compounds are distinctly different from those induced by monofunctional mononuclear platinum(II) compounds, such as [PtCl(dien)]Cl or [PtCl(NH₃)₃]Cl. A major difference between DNA modification by monofunctional platinum compounds and bifunctional dinuclear complexes consists in the ability of the latter to form interstrand cross-links that effectively prevent separation of the complementary strands of DNA. It may be that global conformational changes in the presence of interstrand cross-links will be highly dependent on sequence. The separation of the DNA strands is essential for the processes, such as DNA replication or transcription, so that inhibition of these important processes in tumor cells may represent an important contribution to the cytostatic effects of the dinuclear complexes.

It has been suggested (21, 23) that HMG domain proteins play a role in sensitizing cells to cisplatin. It has been shown that HMG domain proteins recognize and bind to DNA cross-links formed by cisplatin between bases in neighboring base pairs (21–23). The molecular basis for this recognition is still not entirely understood, although several structural details of the 1:1 complex formed between HMG domain and the duplex containing 1,2 d(GpG) intrastrand cross-link were recently elucidated (21). The details of how the binding of HMG domain proteins to cisplatin-modified DNA sensitize tumor cells to cisplatin are also still not completely resolved, but possibilities such as shielding cisplatin-DNA adducts from excision repair or that these proteins could be titrated away from their transcriptional regulatory function have been suggested (23) as clues for how these proteins are involved in the antitumor activity.

An important structural motif recognized by HMG domain proteins on DNA modified by cisplatin is a stable, directional bend of the helix axis (23). As it is demonstrated in the present work and also in our previous paper (12) the major interstrand cross-links and even minor 1,2-d(GpG) intrastrand cross-link of trans-bis-Pt compounds bend helix axis much less efficiently than the cross-links of cisplatin (for instance gel retardation assay revealed that 1,2 d(GpG) intrastrand cross-link of cisplatin produced a rigid, directed bend 32–34° into the major groove of DNA; Ref. 36). Therefore, it was not surprising that we only observed in the present paper (Fig. 7) very weak or no recognition of DNA adducts of trans-bis-Pt complexes by HMG1 protein, consistent with the assumption that an important structural motif recognized by HMG domain proteins is bent or kinked duplex axis. Thus, from the results of the present work, it is clear that the major DNA adducts of antitumor trans-bis-Pt compounds may present a block to DNA or RNA polymerase (14, 19) but are not a substrate for recognition by HMG domain proteins. From these considerations we could conclude that the mechanism of antitumor activity of bifunctional dinuclear platinum complexes does not involve recognition by HMG domain proteins as a crucial step, in contrast to the proposals for cisplatin and its direct analogues.

One possible role for binding of HMG domain proteins to DNA modified by cisplatin is that these proteins shield damaged DNA from intracellular excision repair (23). The examinations of excision repair of DNA modified by dinuclear platinum complexes, which form as major DNA adducts interstrand cross-links, are in progress in our laboratory. In general, DNA interstrand cross-links pose a special challenge to repair enzymes because they involve both strands of DNA and therefore cannot be repaired using the information in the complementary strand for resynthesis. The fact that interstrand cross-links cannot be removed so ready by excision repair as intrastrand lesions is also corroborated by the observation that excision repair of the interstrand cross-link formed by cisplatin was not detected under conditions when intrastrand adducts of this drug were readily removed by a reconstituted system containing highly purified nucleotide excision repair factors (63). Hence, the major DNA adducts of bifunctional dinuclear platinum compounds would not have to be shielded by damaged DNA recognition proteins, such as are those containing HMG domains, to prevent their repair.

In conclusion, the results of the present work provide additional strong support for the hypothesis that platinum drugs that bind to DNA in a fundamentally different manner to that of cisplatin have altered pharmacological properties. Importantly, in contrast to cisplatin, the mediation of antitumor properties of bifunctional dinuclear platinum complexes by HMG domain proteins is unlikely so that polynuclear platinum compounds may represent a novel class of platinum anticancer drugs acting by a different mechanism than cisplatin and its analogues. A further understanding of how bifunctional dinuclear and other polynuclear platinum compounds modify DNA and how these modifications are further processed in cells should provide a rational basis for the design of new chemotherapeutic strategies and platinum antitumor drugs rather than searching for cisplatin analogues.

REFERENCES
1. Chaney, S. G., and Sancar, A. (1996) J. Natl. Cancer Inst. 88, 1346–1360
2. Lokich, J., and Anderson, N. (1998) Ann. Oncol. 9, 13–21
3. O‘Dwyer, P. J., Stevenson, J. P., and Johnson, S. W. (1999) in Cisplatin: Chemistry and Biochemistry of a Leading Anticancer Drug (Lippert, B., ed) pp. 31–72, Verlag Helvetica Chimica Acta, Wiley-VCH, Zurich
4. Johnson, N. P., Butour, J.-L., Villani, G., Wimmer, F. L., Defais, M., Pierson, V., and Brabec, V. (1989) Prog. Clin. Biochem. Med. 10, 1–24
5. Lepre, C. A., and Lippard, S. J. (1990) Nucleic Acids Mol. Biol. 4, 9–38
6. Leng, M., and Brabec, V. (1994) in DNA Adducts: Identification and Biological Significance (Hemminki, K., Dipple, A., Shuker, D. E. G., Kadlubar, F. F., Segerbäck, D., and Bartsh, H., eds) IARC Scientific Publications No. 125, pp. 359–348, International Agency for Research on Cancer, Lyon
7. Blommaert, F. A., van Dijk-Knijnenburg, H. C. M., Dijt, F. J., Denengelse, L., Baan, R. A., Berende, F., and Fichtinger-Schepman, A. M. J. (1995) Biochemistry 34, 8474–8480
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8. Weynarowski, J. M., Chapman, W. G., Napier, C., Herzig, M. C. S., and Juniewicz, P. (1998) Mol. Pharmacol. 54, 770–777
9. Raymond, E., Chaney, S. G., Tsamma, A., and Civitkovic, E. (1998) Ann. Oncol. 9, 1053–1071
10. Farrell, N., Qu, Y., Bierbach, U., Valecchini, M., and Menta, E. (1999) in 30 Years of Cisplatin: Chemistry and Biochemistry of a Leading Anticancer Drug (Lippert, B., ed) pp. 479–496, Verlag Helvetica Chimica Acta, Wiley-VCH, Zurich
11. Farrell, N., Appleton, T. G., Qu, Y., Roberts, J. D., Fintes, A. P. S., Skov, K. A., Wu, P., and Zou, Y. (1995) Biochemistry 34, 15440–15446
12. Kasparkova, J., Melli, K. J., Xu, Y., Brabec, V., and Farrell, N. (1996) Biochemistry 35, 16703–16713
13. Melli, K. J., Xu, Y., Scardale, N., and Farrell, N. (1997) Nucleic Acids Res. 25, 2102–2110
14. Zaludova, R., Zalud, R., Kasparkova, J., Vrana, O., Farrell, N., and Brabec, V. (1997) Eur. J. Biochem. 246, 508–517
15. Kasparkova, J., Novakova, O., Vrana, O., Farrell, N., and Brabec, V. (1999) Biochemistry 38, 10997–11005
16. Brabec, V., Zaludova, R., Vrana, O., Novakova, O., Cox, J. W., Qu, Y., and Farrell, N. (1999) Biochemistry 38, 6781–6790
17. Zou, Y., Van Houten, B., and Farrell, N. (1994) Biochemistry 33, 5404–5410
18. Vrana, O., Boudny, V., and Brabec, V. (1996) Nucleic Acids Res. 24, 3918–3925
19. Zou, Y., Van Houten, B., and Farrell, N. (1994) Biochemistry 33, 5404–5410
20. Zlatanov, J., Yaneva, J., and Leuba, S. H. (1998) FEBS Lett. 12, 791–799
21. Ohndorf, U., Roald, M. A., He, Q., Pabo, C. O., and Lippard, S. J. (1999) Nature 399, 708–712
22. Kasparkova, J., and Brabec, V. (1995) Biochemistry 34, 12379–12387
23. Zamble, D. B., and Lippard, S. J. (1999) in Cisplatin: Chemistry and Biochemistry of a Leading Anticancer Drug (Lippert, B., ed) pp. 73–110, VHCA, WILEY-VCH, Zurich
24. Farrell, N., Qu, Y., Feng, L., and Van Houten, B. (1999) Biochemistry 29, 9522–9531
25. Brabec, V., Reedijk, J., and Leng, M. (1992) Biochemistry 31, 12397–12402
26. Stros, M., and Verlickova, M. (1996) Int. J. Biol. Macromol. 12, 282–288
27. Stros, M., Reich, J., and Kobilavova, A. (1994) FEBS Lett. 344, 201–206
28. Comess, M. K., Costello, C. E., and Lippard, S. J. (1990) Biochemistry 29, 12021–12027
29. Lemaire, M. A., Schwartz, A., Rahmeniu, A. R., and Leng, M. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 1982–1985
30. Maxam, A. M., and Gilbert, W. (1980) Methods Enzymol. 56, 499–560
31. Brabec, V., Sip, M., and Leng, M. (1993) Biochemistry 32, 11676–11681
32. Bailly, C., Gentle, D., Haney, F., Purcell, M., and Waring, M. J. (1994) Biochem. J. 300, 165–173
33. Kasparkova, J., and Burrows, C. J. (1996) Nucleic Acids Res. 24, 5062–5063
34. Bailly, C., and Waring, M. J. (1997) in Drug-DNA Interaction Protocols (Fox, K. R., ed) pp. 51–79, Humana Press Inc., Totowa, NJ
35. Koo, H. S., Wu, H. M., and Broders, D. M. (1986) Nature 320, 501–506
36. Bellon, S. F., and Lippard, S. J. (1990) Biochim. Biophys. Acta. 1053–1071
37. Bianchi, M. E., Beltramme, M., and G. P. (1989) Science 243, 1056–1059
38. Dabrowski, R., Payet, D., and Leng, M. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 8147–8151
39. Lemaire, M., Thuvert, L., Deforesset, B., Viel, A., Beauregard, G., and Potier, M. (1990) Biochem. J. 267, 431–439
40. Nielsen, P. E. (1990) J. Mol. Recog. 3, 1–24
41. McCarthy, J. G., Williams, L. D., and Rich, A. (1990) Biochemistry 29, 6071–6081
42. McCarthy, J. G., and Rich, A. (1991) Nucleic Acids Res. 19, 3421–3429
43. Herr, W. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 8009–8013
44. Johnston, B. H., and Rich, A. (1985) Cell 42, 713–724
45. Koo, H. S., and Crothers, D. M. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 1763–1767
46. Rice, J. A., Crothers, D. M., Pinto, A. L., and Lippard, S. J. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 4158–4161
47. Leng, M. (1990) Biophys. Chem. 35, 155–163
48. Huang, H. F., Zhu, L. M., Reid, R. B., Droby, G. P., and Hopkins, P. B. (1995) Science 270, 1842–1845
49. Bellon, S. F., Coleman, J. H., and Lippard, S. J. (1991) Biochemistry 30, 8026–8035
50. Wang, J. C. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 200–203
51. Rhodes, D., and Klug, A. (1980) Nature 286, 573–578
52. Malinge, J. M., Perez, C., and Leng, M. (1994) Nucleic Acids Res. 22, 3834–3839
53. Zinkel, S., and Broders, D. M. (1987) Nature 326, 178–181
54. Koo, H. S., Drak, J., Rice, J. A., and Crothers, D. M. (1990) Biochemistry 29, 4227–4234
55. Donahue, B. A., Augt, M., Bellon, S. F., Treiber, D. K., Toney, J. H., Lippard, S. J., and Essigmann, J. M. (1990) Biochemistry 29, 5872–5880
56. Pil, P. M., and Lippard, S. J. (1992) Science 256, 234–237
57. Yang, D. Z., Vanboom, S. S. G. E., Reedijk, J., Vanboom, J. H., and Wang, A. H. J. (1995) Biochemistry 34, 12912–12920
58. Takahara, P. M., Frederick, C. A., and Lippard, S. J. (1996) J. Am. Chem. Soc. 118, 12309–12321
59. Gelasco, A., and Lippard, S. J. (1998) Biochemistry 37, 9230–9239
60. Brabec, V., Boudny, V., and Balcarova, Z. (1994) Biochemistry 33, 1316–1322
61. Keck, M. V., and Lippard, S. J. (1992) J. Am. Chem. Soc. 114, 3386–3390
62. Wu, P. K., Kharatishvilii, M., Qu, Y., and Farrell, N. (1996) J. Inorg. Biochem. 63, 9–18
63. Zamble, D. B., Mu, D., Beardon, J. T., Sancar, A., and Lippard, S. J. (1996) Biochemistry 35, 10004–10013