Chemistry & Biology, Vol. 9, 629–638, May, 2002, ©2002 Elsevier Science Ltd. All rights reserved. PII S1074-5521(02)00134-5

Recognition of Major DNA Adducts of Enantiomeric Cisplatin Analogs by HMG Box Proteins and Nucleotide Excision Repair of These Adducts

Jaroslav Malina,[1] Jana Kasparkova,[1] Giovanni Natile,[2] and Viktor Brabec[1,3]
[1]Institute of Biophysics
Academy of Sciences of the Czech Republic
Kralovopolska 135
CZ-61265 Brno
Czech Republic
[2]Department of Pharmaceutical Chemistry
University of Bari
I-70125 Bari
Italy

Summary

We examined HMG domain protein recognition of major 1,2-GG intrastrand DNA crosslinks, formed by two bifunctional enantiomeric analogs of antitumor *cis*-diamminedichloroplatinum(II) (cisplatin), and removal of these crosslinks during in vitro nucleotide excision repair (NER) reactions. Electrophoretic mobility shift assays show that domains A and B of HMGB1 protein bind to (2*R*,3*R*)-diaminobutanedichloroplatinum(II)-generated crosslinks with a higher affinity than to those generated by (2*S*,3*S*)-diaminobutanedichloroplatinum(II). The crosslinks of both enantiomers are removed by NER with a similar efficiency; however, HMG1B protein significantly inhibits removal of the (2*R*,3*R*)-diaminobutaneplatinum(II) adduct, but not that of the (2*S*,3*S*) enantiomer. Thus, HMG domain proteins discriminate among different conformations of the 1,2-GG intrastrand crosslinks of the two enantiomeric analogs of cisplatin, which results in different NER of these crosslinks. This observation may provide insight into the mechanisms underlying antitumor activity of cisplatin and its analogs.

Introduction

The mechanism of antitumor activity of *cis*-diamminedichloroplatinum(II) (cisplatin) is of continuing interest because understanding it may help in designing new platinum drugs with better or alternative therapeutic properties. One concept of designing new platinum drugs is based on the observation that carrier amine ligands of cisplatin can modulate its anticancer properties. For instance, activity is usually lost or diminished if the primary or secondary amines on platinum are replaced by tertiary amines [1]. Cisplatin reacts with a number of cellular components, but it is generally accepted that the biologically relevant target is DNA [2]. Cisplatin preferentially reacts with N7 atoms of neighboring purines in DNA (either two guanines or adenine and guanine), giving rise to 1,2-GG intrastrand crosslinks (CLs). The dependence of the activity of cisplatin analogs on the character of carrier ligands has led to the hypothesis that O6-NH [3–6] and/or phosphate-NH intramolecular hydrogen bonds within the 1,2-intrastrand CL affect DNA conformation and hence activity of the platinum compound [4–12].

Interactions between DNA and carrier ligands of platinum compounds could affect the initial attack of the drug on DNA and the type of CL (intra- or interstrand) as well as the direction of the crosslinking reaction (toward the 3′ or 5′ end of the DNA strand). The carrier ligand may also affect, for instance, pharmacokinetics and distribution of platinum drugs in tumor cells along with recognition of damaged DNA by repair enzymes or other damaged-DNA binding proteins, which may affect the further processing of DNA adducts in tumor cells.

Because DNA (and several other cellular substrates) is a chiral molecule, it may interact in a different way with the platinum complexes containing enantiomeric amine ligands such as ((1*R*,2*R*)-diaminocyclohexane)-platinum(II) and ((1*S*,2*S*)-diaminocyclohexane)platinum(II) [Pt(*R,R*-DACH) and Pt(*S,S*-DACH), respectively; DACH = 1,2-diaminocyclohexane] [13–15]. Although Pt(*R,R*-DACH) and Pt(*S,S*-DACH) produce the same kind of intra- and interstrand DNA CLs (which are similar to those formed by cisplatin) [16], the biological activity of the two enantiomers is different [17–26]. Generally, the differences in biological activity of enantiomeric forms of platinum(II) compounds with chiral amine ligands are not so pronounced unless the rotational freedom of the asymmetric center is reduced by placing, for instance, the chiral center(s) on the carbon chain linking two nitrogen atoms of a chelate diamine [17–20, 22, 25, 26]. Thus, for instance, it has been found [17–20, 22, 25, 26] that Pt(*R,R*-DACH) exhibits higher antitumor activity than Pt(*S,S*-DACH).

Chiral carbon atoms in the chain bridging the two nitrogens influence both the puckering of the chelate ring (particularly in the case of five-membered chelate rings) and the stereochemistry on the coordinated nitrogens with one "quasi axial" and one "quasi equatorial" hydrogen. There is a relationship between antitumor efficiency of the platinum compound and the conformation of the five-membered chelate ring of the diamine in the carrier ligand of the platinum drug. Usually, the compounds with a λ-gauche conformation of the chelate ring exhibit higher antitumor activity than those having a δ-gauche conformation [19]. In contrast, compounds with a δ-gauche conformation of the chelate ring of the diamine exhibit higher mutagenic activity which, in some cases, can be even ten times greater than that of the other enantiomer [22]. As a result of the markedly different behavior of the two enantiomeric forms, only ((1*R*,2*R*)-diaminocyclohexane)oxalatoplatinum(II) (oxaliplatin) (having a λ-gauche conformation of the chelate ring) and not the *S,S* enantiomer has been approved in Europe, Latin America, and Asia for clinical use [27]. Hence, studies have mainly focused on DNA modifications by the complex having the *R,R*-DACH ligand. The recently reported [28, 29] crystal structure of 1,2-GG intrastrand CL formed by oxaliplatin in a DNA dodeca-

[3]Correspondence: brabec@ibp.cz

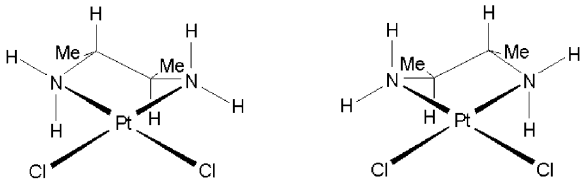

Figure 1. Structures of the Platinum Compounds Tested in the Present Study

nucleotide duplex has shown that the overall geometry is similar to that of cisplatin. A novel feature of this structure is the presence of a hydrogen bond between the pseudoequatorial NH hydrogen atom of the *R*,*R*-DACH ligand and the O6 atom of the 3′-G of the CL. This finding has confirmed the importance of chirality in mediating the interaction between cisplatin analogs containing enantiomeric amine ligands and double-helical DNA. In addition, a very recent report [30] has demonstrated that HMGB1 and TATA binding proteins recognize 1,2-GG intrastrand CL of Pt(*R,R*-DACH).

Several attempts have been made to find relationships between the configuration of the carrier ligands of the platinum complexes and structural modifications induced in DNA by these compounds and/or cellular processing of the resulting DNA damage. We have shown in our recent work [31] that 1,2-d(GpG) intrastrand CL of ((2*R*,3*R*)-diaminobutane)platinum(II) or ((2*S*,3*S*)-diaminobutane)platinum(II) [Pt(*R*,*R*-DAB) or Pt(*S*,*S*-DAB), respectively; DAB = 2,3-diaminobutane] (Figure 1) not only destabilize DNA differently but also bend and unwind DNA to a different extent. DNA containing platinum adducts that induce stable directional bending and unwinding attracts various damaged-DNA binding proteins, such as those containing the high-mobility group (HMG) domain [32–35]. The affinity of these proteins to 1,2-GG intrastrand CLs of cisplatin depends on several factors and the efficiency of the adducts to thermodynamically destabilize DNA is among the most important. The binding of these proteins has been postulated to mediate the antitumor properties of the platinum drugs [34, 35]. In addition, several reports [36–38] have demonstrated that intrastrand CLs of cisplatin and its direct analogs are removed from DNA during nucleotide excision repair (NER) reactions and that NER is also an important mechanism contributing to cisplatin resistance.

Therefore, in order to shed light on how chirality at the carbon atoms of the carrier ligand in cisplatin analogs can affect processing its major adducts in cells, we investigated in the present work how HMGB1 box proteins and the NER differentiate between major DNA adducts of cisplatin analogs having enantiomeric nonleaving ligands during in vitro reactions. We have chosen for these studies Pt(*R*,*R*-DAB) and Pt(*S*,*S*-DAB) compounds as the representatives of cisplatin analogs with enantiomeric amine ligands because the effect of chirality at the carbon atoms on the biological activity was most pronounced in the case of this class of cisplatin analogs [22]. Thus, the results of the present work may also serve to clarify the role of enantiomorphism of carrier ammine ligands in oxaliplatin, the second-generation platinum anticancer drug already used in the clinic.

Results

Recognition by the Domains A and B of HMGB1

To determine the effect of chirality at the carbon atoms of the carrier ligand in cisplatin analogs on the recognition of platinated DNA by HMGB1 box proteins, we investigated the interactions of the rat HMGB1 domain A (HMGB1a) and HMGB1 domain B (HMGB1b) with 1,2-GG intrastrand CLs of Pt(*R,R*-DAB) or Pt(*S,S*-DAB). In these experiments, the 20 base pair (bp) duplex TGGT/AGGA(1) (Table 1) was modified so that it contained a single, site-specific 1,2-GG intrastrand CL of Pt(*R,R*-DAB), Pt(*S,S*-DAB), or, for comparative purposes, also that of cisplatin. The binding of HMGB1a and HMGB1b to these DNA probes was detected by retardation of the migration of the radiolabeled 20 bp probes through the gel [34, 39, 40] (Figure 2).

HMGB1a and HMGB1b exhibited negligible binding to the nonmodified 20 bp duplex. As indicated by the presence of a shifted band whose intensity increases with increasing protein concentration (shown for HMGB1a in Figure 2A), both HMGB1a and HMGB1b recognize the duplex containing the 1,2-GG intrastrand CL of either Pt(*R,R*-DAB) or Pt(*S,S*-DAB). Because only a single-shifted band forms after incubation of TGGT/AGGA(1) duplex containing the CL with either HMGB1a or HMGB1b, detailed titration studies were possible. The results of the titration of this duplex containing the 1,2-GG intrastrand CL of either Pt(*R,R*-DAB) or Pt(*S,S*-DAB) with HMGB1a or HMGB1b are shown in Figures 2B and 2C, respectively. Evaluations of these titration data afforded the $K_{D(app)}$ values reported in Table 2. These titration data indicate that HMGB1a binds the probe containing the CL of Pt(*R*,*R*-DAB) with a relatively high

Table 1. Oligodeoxyribonucleotide Sequences and Abbreviations

Abbreviation	Sequence
TGGT[a]	5′-CCTCTCCTT**GG**TCTTCTTCT-3′
ACCA(1)	5′-AGAAGAAGACCAAGGAGAGG-3′
ACCA(2)	5′-GCTCAGAAGAAGACCAAGGAGAGGTCAG-3′
64-mer(1)	5′-GGGACCTGAACACGTACGGAATTCGATATCCTCGAGCCAGATCTGCGCCAGCTGGCCACCCTGA-3′
64-mer(2)	5′-GAGCGCCAAGCTTGGGCTGCAGCAGGTCGACTCTAGAGGATCCCGGGCGAGCTCGAATTCGCCC-3′
61-mer	5′-GGGGCGAATTCGAGCTCGCCCGGGATCCTCTAGAGTCGACCTGCTGCAGCCCAAGCTTGGC-3′
59-mer	5′-GGTGGCCAGCTGGCGCAGATCTGGCTCGAGGATATCGAATTCCGTACGTGTTCAGGTCC-3′

[a] Bold face indicates the sites of platination.

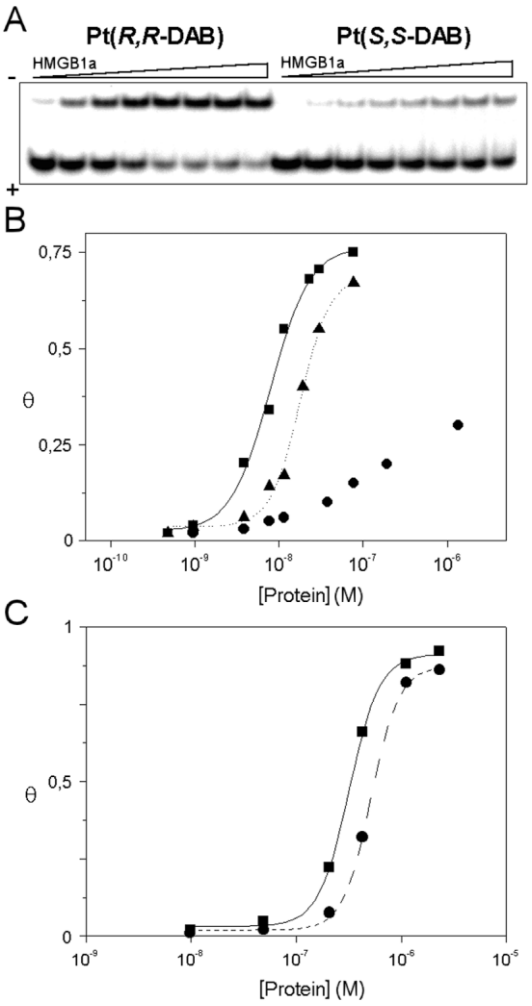

Figure 2. Gel Mobility Shift Assay Analysis of the Titration of the 20 bp Duplex TGGT/ACCA(1) Containing the Single 1,2-GG Intrastrand CL of Pt(*R*,*R*-DAB) or Pt(*S*,*S*-DAB) with HMGB1a and HMGB1b

(A) Autoradiogram of gel mobility shift assay of the titration with HMGB1a (0.5 nM–1.4 μM). The concentration of the 20 bp duplex was 10 nM.

(B) Plots of the fraction of bound DNA (θ) versus HMGB1a concentration with superimposed fits to the equation $\theta = P/P + K_{D(app)}$ (*P* is the total protein concentration, and $K_{D(app)}$ is the apparent dissociation constant) [41, 71]. DNA probes were the 20 bp duplex TGGT/ACCA(1) (10 nM) containing the single 1,2-GG intrastrand CL of Pt(*R*,*R*-DAB) (squares), Pt(*S*,*S*-DAB) (circles) or cisplatin (triangles).

(C) Plots of the fraction of bound DNA (θ) versus HMGB1b concentration with superimposed fits to the equation given in the legend to Figure 2B. Other details were the same as in Figure 2B.

affinity, which was even higher than that of HMGB1a to the same probe containing the CL of cisplatin. On the other hand, the binding of HMGB1a to the probe containing the CL of Pt(*S*,*S*-DAB) was considerably reduced; for instance, the fraction of bound oligonucleotide probe was only ~0.3 at a protein concentration as high as 1 μM. Because a relatively high amount of the HMGB1a protein would be needed to complete the titration experiment with the probe containing the CL of Pt(*S*,*S*-DAB), this binding was also investigated via a competition assay (Figure 3). Addition of the unlabeled

Table 2. $K_{D(app)}$ Values Determined for HMGB1 Box Protein Interactions with 20 bp Oligonucleotide Duplexes TGGT/ACCA(1) Containing Single 1,2-GG Intrastrand Crosslink of Pt(*R*,*R*-DAB), Pt(*S*,*S*-DAB), or Cisplatin

Duplex	HMGB1a	HMGB1b
20 bp/*R*,*R*[a]	11 ± 2 7 ± 1[d]	320 ± 34
20 bp/*S*,*S*[b]	113 ± 19[d]	510 ± 60
20 bp/cisplatin[c]	26 ± 3 14 ± 2[d]	

$K_{D(app)}$ values are in nM and were measured by direct titration unless otherwise specified.

[a] The duplex containing the adduct of Pt(*R*,*R*-DAB).

[b] The duplex containing the adduct of Pt(*S*,*S*-DAB).

[c] The duplex containing the adduct of cisplatin.

[d] Measured from competition experiments.

duplex TGGT/ACCA(1) containing the CL of either enantiomer or cisplatin competes with the binding of HMGB1a to labeled duplex TGGT/ACCA(1) containing the 1,2-GG intrastrand CL of cisplatin (shown in Figure 3A for the unlabeled probe containing the CL of Pt(*R*,*R*-DAB)). A plot quantitating these competition data is presented in Figure 3B. The apparent dissociation constants for the competitors, such as duplexes containing the 1,2-GG intrastrand CL of Pt(*R*,*R*-DAB), Pt(*S*,*S*-DAB), or cisplatin were determined as described [41] and are given in Table 2. These data confirmed that HMGB1a

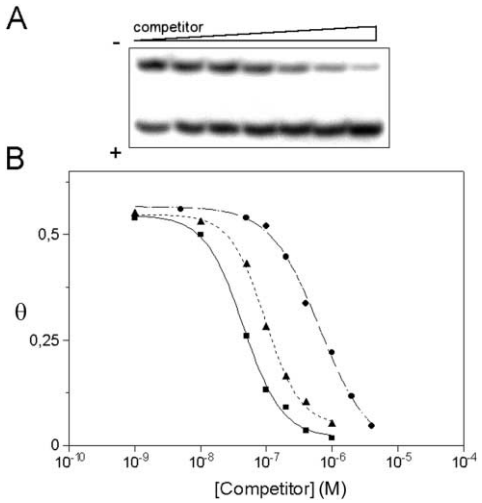

Figure 3. Analysis of the Self-Competition of the Complex of HMGB1a with the 20 bp Duplex TGGT/ACCA Containing the 1,2-GG Intrastrand CL of Cisplatin with Unlabeled Competitor TGGT/ACCA(1) Containing the 1,2-GG Intrastrand CL of Pt(*R*,*R*-DAB)

(A) Gel mobility shift assay. The 20 bp duplex TGGT/ACCA was at a concentration of 10 nM.

(B) Plot of the fraction of bound DNA θ versus competitor concentration for unlabeled TGGT/ACCA(1) competitors containing the 1,2-GG intrastrand CL of Pt(*R*,*R*-DAB) (squares), Pt(*S*,*S*-DAB) (circles), or cisplatin (triangles) with the duplex containing the 1,2-GG intrastrand CL of cisplatin. Fits of the data to the equation $\theta = 1/2T_t\{K_t + K_{rel}C_t + P_t + T_t - [(K_t+K_{rel}C_t+P_t+T_t)^2 - 4P_tT_t]^{1/2}\}$ (K_{rel} is the ratio of K_t [the apparent dissociation constant of the labeled probe] to the apparent dissociation constant of the competitor, and P_t, T_t, and C_t are the concentrations of protein, radiolabeled probe, and competitor probe, respectively [41, 72]) are superimposed.

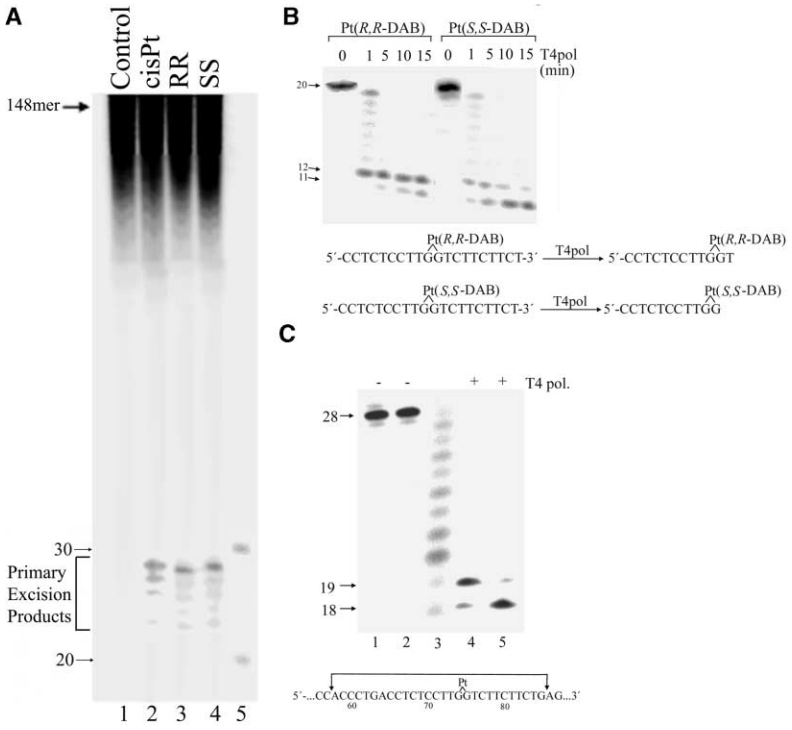

Figure 4. Excision of 1,2-GG Intrastrand Crosslinks of Pt(*R*,*R*-DAB), Pt(*S*,*S*-DAB), or Cisplatin by Rodent Excinuclease

(A) Substrates (148 bp) containing the central and unique intrastrand CL of Pt(*R*,*R*-DAB) (lane RR), Pt(*S*,*S*-DAB) (lane SS), or cisplatin (lane cisPt) were incubated with CHO CFE and resolved in 10% denaturing polyacrylamide gel. Excision products released during the reaction are primarily 23–28 nucleotides in length and are not observed in the absence of CFE (not shown). In this experiment, the percentages of excision by CHO CFE were around 7%.

(B) Mapping of incision sites. Time course analysis with T4 DNA polymerase and 20-mer duplexes containing the 1,2-GG intrastrand CL of Pt(*R*,*R*-DAB) or Pt(*S*,*S*-DAB) formed at the central sequence TGGT were used to identify sites of inhibition of T4 DNA polymerase exonuclease activity. At all time points, this exonuclease activity was primarily blocked at the second or first nucleotide 3′ to the CL of Pt(*R*,*R*-DAB) or Pt(*S*,*S*-DAB), respectively, resulting in the migration of platinated 20-mers as platinated 12- or 11-mers.

(C) Limited (10 min) T4 DNA polymerase digestion was used to identify the 3′ incision site of gel-purified oligomers released during the excision repair reaction (Figure 4A, lanes RR and SS). The excised 28-mers containing the CL of Pt(*R*,*R*-DAB) (lane 1) or Pt(*S*,*S*-DAB) (lane 2) migrated as a 19-mer (lane 4) or 18-mer (lane 5), respectively, after treatment with T4 DNA polymerase. Lane 3 contains the mixture of single-stranded oligodeoxyribonucleotides 18–28 nucleotides long as the markers. Thus, in the case of both CLs, one incision occurs at the 11th phosphodiester bond 3′ to the CL, and the second incision occurs at the 17th bond on the 5′ side to generate a 28-mer excision product. For other details, see the text.

binds to the 1,2 GG intrastrand CL of Pt(*R,R*-DAB) with approximately two times or 16 times higher affinity than was that to the CL of cisplatin or Pt(*S,S*-DAB), respectively.

The titration of the 20-bp duplex containing the 1,2-GG intrastrand CL of Pt(*R*,*R*-DAB) or Pt(*S*,*S*-DAB) with HMGB1b has revealed (Figure 2B) that this protein also binds to the intrastrand CL of these platinum compounds, although with considerably less affinity (∼30 times less) than that with which HMGB1a binds to the probes crosslinked by Pt(*R*,*R*-DAB) or cisplatin. HMGB1b binds to the probes that are intrastrand crosslinked by both enantiomers with approximately the same affinity, although the affinity to the CL of Pt(*S*,*S*-DAB) was slightly lower.

Nucleotide Excision Repair

NER is a pathway used by human cells for the removal of damaged nucleotides from DNA [42–44]. In mammalian cells, this repair pathway is an important mechanism for the removal of bulky, helix-distorting DNA adducts, such as those generated by various chemotherapeutics, including cisplatin [45]. Efficient repair of 1,2-GG and 1,3-GTG intrastrand CLs of cisplatin by various NER systems, including human and rodent excinucleases, has been reported [36–38, 46–48]. The result presented in Figure 4A, lane 2 confirms these reports. Importantly, 1,2-GG intrastrand CL of Pt(*R*,*R*-DAB) or Pt(*S*,*S*-DAB) was also repaired with a similar efficiency by both human and rodent excinucleases (shown for the CLs repaired by rodent excinuclease in Figure 4A, lanes RR and SS).

The NER assay detects radiolabeled fragments resulting from dual incisions both 5′ and 3′ to the lesion. The length of the major excision products, generated by NER of the 1,2-GG intrastrand CLs of Pt(*R*,*R*-DAB) or Pt(*S*,*S*-DAB) (Figure 4A, lanes RR and SS) was determined in the following way. The excised fragments were cut off from the gel, isolated, and purified, and their length was determined by a comparison of their electrophoretic mobility in the gel with the mobility of the unplatinated marker oligodeoxyribonucleotides. This determination is, however, complicated by the fact that the mobility of the excised fragments is affected by the 2+ charge of the platinum complex moiety. The decreased mobility of the excised platinated fragments was, therefore, reversed by NaCN treatment (0.2 M, pH 10–11, 45°C overnight), which removes platinum from DNA. The following analysis revealed that the major excision fragment contained 28 nucleotides and that the other primary excision fragments were 23–27 nucleotides in length (vide supra), although 22–31 nucleotide-long fragments were also observed (Figure 4A, lanes 2–4). This range of product sizes reflects variability at both the 3′ and 5′ incision sites [37, 49]; smaller excision products are due to degradation of the primary excision products by exonucleases present in the extracts [37].

T4 DNA polymerase 3′→5′ exonuclease activity was used to map the primary sites of incision. If a DNA adduct terminates the exonuclease activity so that it is a block to this activity, limited digestion with the exonuclease demonstrates that the damaged nucleotide(s) is in the excised oligomer. In addition, the limited digestion can

also be used to map the 3′ incision site. Incubation of the 20-mer TGGT containing the single 1,2-GG intrastrand CL of either DAB enantiomer (used to prepare the 148 bp duplex as the substrate for the nucleotide excision assay) with T4 DNA polymerase in the absence of deoxyribonucleotide triphosphates (i.e., exploiting 3′→5′ exonuclease activity of this polymerase) was used to determine the nucleotide at which the exonuclease activity is inhibited. Interestingly, after incubation of the 20-mer oligonucleotides containing the CL of Pt(*R*,*R*-DAB) or Pt(*S*,*S*-DAB) (10 min) with T4 DNA polymerase under these conditions, the resulting products migrated as a species 8 or 9 nucleotides less than the starting material (Figure 4B), indicating that the exonuclease activity was blocked at the first nucleotide 3′ to the intrastrand CL or directly at the 3′ guanine of the CL, respectively.

When the 28-mer excision product, generated by repair of the 1,2-GG intrastrand CL of Pt(*R*,*R*-DAB) or Pt(*S*,*S*-DAB), was treated in the same manner, it was shortened by 9 or 10 nucleotides, respectively (Figure 4C). Because the data with the 20-mer show that T4 DNA polymerase 3′→5′ exonuclease activity stops one nucleotide 3′ to the CL or directly at the 3′ guanine of the CL, they imply that the 3′ incision site is 10 nucleotides (or at the 11th phosphodiester bond) 3′ to the CL of both DAB enantiomers. This in turn places the other incision site at the 17th phosphodiester bond 5′ to the CL of both enantiomers.

Inhibition of Nucleotide Excision Repair by Full-Length HMGB1 Protein

It has been shown [36, 50] that various HMGB1 proteins specifically inhibit NER of the 1,2-GG or AG intrastrand CL of cisplatin. In addition, we have shown in the present work that HMGB1a, which is the dominating domain in full-length HMGB1 that binds to the site of the 1,2 intrastrand CL of cisplatin [40, 51], binds with a markedly higher affinity to the CL of Pt(*R*,*R*-DAB) than to that of its *S,S* enantiomer. When the excision assay was conducted by the addition of rodent cell-free extract (CFE) to the reaction mixtures containing HMGB1 and crosslinked DNA, excision of the 1,2-GG intrastrand CL of Pt(*R*,*R*-DAB) was monotonically inhibited with increasing protein concentration, whereas excision of the 1,2-GG intrastrand CL of Pt(*S*,*S*-DAB) was not affected by HMGB1 addition at the concentrations up to ~19 μM and was then inhibited at higher concentrations (Figure 5). Hence, consistent with the higher affinity of the HMGB1a to the 1,2-GG intrastrand CL of Pt(*R*,*R*-DAB) (Figure 2; Table 2), excision of this CL was inhibited by HMGB1 markedly more than that of the CL of its *S,S* enantiomer (Figure 5). In comparison with that of the *S,S* enantiomer, inhibition of NER of the 1,2-GG intrastrand CL of the *R,R* enantiomer by HMGB1 was markedly higher in HeLa CFE as well (our unpublished data). The fact that NER of the 1,2-GG intrastrand CL of Pt(*R*,*R*-DAB) is blocked by HMGB1 at concentrations of protein that do not inhibit NER of the 1,2-GG intrastrand CL of Pt(*S*,*S*-DAB) is consistent with the view that this effect is not a consequence of nonspecific inhibition of the repair factors by this protein [36].

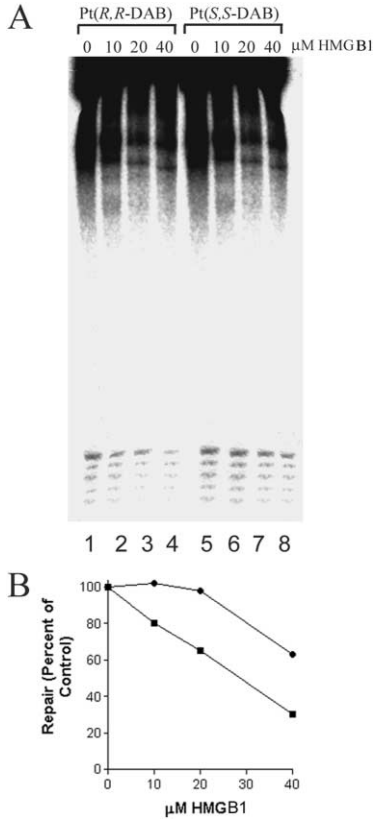

Figure 5. Effect of HMGB1 on NER of the 1,2-GG Intrastrand CL of Pt(*R*,*R*-DAB) or Pt(*S*,*S*-DAB) by Rodent Excinuclease

(A) Denaturing 10% polyacrylamide gel showing inhibition of excision of the CLs of Pt(*R*,*R*-DAB) (lanes 1–4) or Pt(*S*,*S*-DAB) (lanes 5–8). The 148 bp substrates containing the single and central CL were incubated with the indicated concentrations of HMGB1 for 1 hr on ice under NER assay conditions prior to the addition of the rodent CFE and further incubation for 40 min.

(B) Plot of four NER experiments such as those shown in panel A. The 148 bp substrates 1,2-GG crosslinked by Pt(*R*,*R*-DAB) (squares) or Pt(*S*,*S*-DAB) (circles). Data points are averages of three experiments.

Discussion

As shown for the 1,2-GG intrastrand CL of cisplatin [31], HMGB1a binds to DNA around the site of this adduct in the minor groove, whereas the amine ligands coordinated to platinum reside in the major groove. It is therefore unlikely that the different affinity of HMGB1a to the 1,2-GG intrastrand CLs of Pt(*R*,*R*-DAB) and Pt(*S*,*S*-DAB) is due to different direct interactions of the enantiomeric nonleaving ligands with the protein. Some structural features of DNA containing the 1,2-GG intrastrand CL of Pt(*R*,*R*-DAB) and Pt(*S*,*S*-DAB) have been determined and have revealed considerable differences [31]. The most striking difference is DNA bending induced by these adducts. Whereas the 1,2-GG intrastrand CL of Pt(*R*,*R*-DAB) bends DNA only slightly more than cisplatin (the bending angles determined by a phasing assay were 35° or 32–34° for the CLs of Pt(*R*,*R*-DAB) or cisplatin, respectively), the CL of the *S,S* enantiomer bends DNA considerably less (24°) [31]. Thus, this result is consistent

with the view that *S,S*-DAB ligand could restrict the bending angle of the 1,2-GG intrastrand CL. In addition, when HMGB1a binds to the 1,2-GG intrastrand CL of cisplatin, the protein induces further bending of the DNA [34, 35]. Hence, it is also possible that the bulky DAB group in the 1,2-GG intrastrand CL of Pt(*S,S*-DAB) restricts the additional DNA bending, required for HMGB1a binding, markedly more than in the CL of Pt(*R,R*-DAB) or cisplatin.

Recently published results [28, 29] indicate several very distinct properties of the 1,2-GG intrastrand CLs formed by cisplatin and Pt(*R,R*-DACH) [this latter compound is closely related to Pt(*R,R*-DAB) examined in the present work]. Although the hydrogen bond between 5′ phosphate and cisplatin ammonia oriented toward 5′ guanine in the platinated site is readily formed, this type of hydrogen bond does not occur if cisplatin in this CL is replaced by Pt(*R,R*-DACH). On the other hand, a new chiral interaction is observed in the CL formed by Pt(*R,R*-DACH). This new interaction involves the hydrogen bonding between the pseudo-equatorial NH of the *R,R*-DACH ligand and the O6 atom of the 3′ guanine of the platinated site. It is reasonable to expect that the hydrogen bonding in the CLs formed by Pt(*R,R*-DAB) and Pt(*R,R*-DACH) is similar [52, 53]. In addition, the results of the present work (Figures 2A and 2B) demonstrate that the affinity of HMGB1a to the 1,2-GG intrastrand CLs of cisplatin and Pt(*R,R*-DAB) is also similar. Therefore, hydrogen bonds between 5′ cisplatin ammonia and 5′ phosphate in the platinated site or between the pseudo-equatorial NH of the *R,R*-DAB ligand and the O6 atom of the 3′ guanine of the platinated site may not substantially affect the affinity of HMGB1a for the 1,2-GG intrastrand CL of cisplatin or its analogs. In contrast, it has been proposed for the CL of Pt(*S,S*-DAB) [52, 53] that a hydrogen bond forms between the pseudo-equatorial NH of the *S,S*-DAB ligand and the O6 atom of the 5′ guanine of the platinated site. The latter hydrogen bond and resulting conformational distortion in DNA are not observed if the 1,2-GG intrastrand CLs of cisplatin or *R,R* enantiomer is formed. This is consistent with the view that the hydrogen bond between the pseudo-equatorial NH of the *S,S*-carrier ligand and the O6 atom of the 5′ guanine of the platinated site, and perhaps also the more extensive distortion on the 5′ side of the CL formed by the *S,S* enantiomer, are the factors that reduce the affinity of this CL to HMGB1a.

It has been shown recently [54, 55] that the reduced binding affinity of HMGB1a to the 1,2-GG intrastrand CL of platinum compounds correlates with an enhanced CL-induced energetic destabilization of the host duplex. In our recent work [31], we have also analyzed by differential scanning calorimetry the 20-bp duplexes containing the single 1,2-GG intrastrand CL of Pt(*R,R*-DAB) or Pt(*S,S*-DAB). The CL of Pt(*S,S*-DAB) resulted in a higher destabilization of the 20 bp host duplex than the CL of its *R,R* enantiomer, and consistently, the affinity of HMGB1a to the CL of Pt(*S,S*-DAB) is reduced (Figures 2 and 3).

Further molecular biophysical studies are required to determine whether the hydrogen bond between the NH of the *S,S*-DAB ligand and the O6 atom of the 5′ guanine of the 1,2-GG intrastrand CL of Pt(*S,S*-DAB), the restricted DNA bending, or some other features result in the reduced affinity of HMGB1a for this adduct.

As mentioned above, it is also possible that the different affinity of the HMGB1a for the 1,2-GG intrastrand CLs of Pt(*R,R*-DAB) and Pt(*S,S*-DAB) is also associated with the observation that the CL of Pt(*S,S*-DAB) induces a greater distortion on the 5′ side of the adduct than its *R,R* enantiomer [31]. The distortion on the 5′ side may occur because of a new chiral interaction of the *S,S*-DAB ligand. Thus, it seems reasonable to suggest that the more pronounced distortion on the 5′ side of the CL reduces affinity of the HMGB1a to the 1,2-GG intrastrand CLs of cisplatin analogs. On the other hand, the CL of Pt(*R,R*-DAB) induces a greater distortion on the 3′ side of the adduct [31]. Interestingly, HMGB1a makes contacts with DNA containing the 1,2-GG intrastrand CL of cisplatin asymmetrically and mainly on the 3′ side of the CL [40]. Thus, the latter result is consistent with the somewhat surprising hypothesis that the distortion on the 3′ side of the CL formed by the *R,R* enantiomer does not markedly affect the binding of HMGB1a protein to the intrastrand CL of Pt-DAB compounds.

Several reports [36–38] have demonstrated that NER is a major mechanism contributing to cisplatin resistance and that the binding of HMG domain proteins to 1,2-GG intrastrand CLs has been postulated to mediate antitumor properties of the platinum drugs [34, 35]. The details of how the binding of HMG domain proteins to cisplatin-modified DNA sensitizes tumor cells to cisplatin are still not completely resolved, but possibilities such as the shielding of platinum-DNA adducts from NER or the titration of these proteins away from their transcriptional regulatory function have been suggested [35, 56–58] as clues for how these proteins are involved in the antitumor activity. Our results (Figure 4A) demonstrate that the 1,2-GG intrastrand CLs of Pt(*R,R*-DAB) or Pt(*S,S*-DAB) are repaired by the NER system in vitro with a similar efficiency. On the other hand, HMGB1 protein interferes with repair of the 1,2-GG intrastrand CL of Pt(*R,R*-DAB) considerably more than with that of the CL of Pt(*S,S*-DAB) (Figure 5). Hence, it is possible to suggest that the stereochemistry of the carrier amine ligands of cisplatin analogs significantly, although indirectly, influences repair of their major 1,2-intrastrand CLs and, in this way, also the toxicity of the platinum compounds toward cancer cell lines.

It has been suggested that during replication, translesion synthesis past platinum adducts catalyzed by DNA polymerases may be error prone and result in mutations. No data that would allow comparison of translesion synthesis past the 1,2-GG intrastrand CL of Pt(*R,R*-DAB) and Pt(*S,S*-DAB) are available. On the other hand, it has been shown [59, 60] that translesion synthesis past the 1,2-GG intrastrand CL of cisplatin or oxaliplatin is markedly or entirely inhibited if this CL is bound to HMGB1 protein. Hence, it is reasonable to expect that HMGB1 proteins can modulate the efficiency of DNA polymerases to bypass the 1,2-GG intrastrand CL also formed by Pt-DAB compounds and can consequently affect their mutagenicity. Thus, considerably lower mutagenic effects of Pt(*R,R*-DAB) in comparison with Pt(*S,S*-DAB) [59, 60] could be due to markedly tighter binding of HMG domain proteins to the major 1,2-GG intrastrand CL of

Pt(*R*,*R*-DAB) than to the CL of Pt(*S*,*S*-DAB); this tighter binding would inhibit translesion synthesis past the CL more efficiently and consequently would reduce its mutagenic effects.

This work reveals that enantiomorphism of amine ligands affects the specific recognition by HMG box proteins and NER of major DNA intrastrand CL of antitumor analogs of cisplatin. Electrophoretic mobility shift assays show that both the A domain of HMGB1 or full-length HMGB1 discriminate between the 1,2-GG intrastrand CLs of the two enantiomers Pt(*R*,*R*-DAB) and Pt(*S*,*S*-DAB). The results of the present work suggest that this discrimination is responsible at least partly for different processing of these CLs by cellular components, such as recognition by DNA binding proteins and repair, i.e., the processes that play a crucial role in the mechanisms underlying antitumor and mutagenic activity of platinum compounds. The effects of enantiomeric amine ligands have been investigated in the present work for the 1,2-GG intrastrand CL flanked by two thymine residues. The possibility remains that the sequence context may also play a role in discrimination between the intrastrand CLs of both enantiomers of Pt-DAB compounds by DNA binding proteins or by components of the repair systems. Work is in progress to examine the importance of sequence context on both the conformation of DNA containing major 1,2-GG intrastrand CL of cisplatin analogs with enantiomeric amine ligands and the recognition and repair of this DNA.

Significance

Cisplatin is one of the most effective antitumor drugs. Its clinical efficacy is, however, limited. Recently, several new platinum compounds that demonstrate promising antitumor activities, including activity in cisplatin-resistant tumors, were synthesized. One group of these compounds involves analogs of "classical" cisplatin containing enantiomeric amine ligands; of this class, the first compound already used in the clinic is oxaliplatin [((1*R*,2*R*)-diaminocyclohexane)oxalatoplatinum(II)]. Interestingly, the *S*,*S enantiomer* of oxaliplatin exhibits a markedly reduced antitumor activity. Thus, the stereochemistry of carrier amine ligands of cisplatin analogs can modulate their anticancer properties, but the underlying mechanisms have not been entirely clarified. The rational design of cisplatin analogs containing enantiomeric amine ligands and exhibiting improved or alternative antitumor properties requires, therefore, an understanding of the molecular rules governing the effects of enantiomorphism of amine ligands in cisplatin analogs on their biological efficacy. The pharmacologically relevant target of platinum compounds is DNA. Our present study has clarified the role of different conformations of the major 1,2-GG intrastrand crosslinks formed in DNA by two cisplatin analogs containing enantiomeric (2*R*,3*R*)- and (2*S*,3*S*)-diaminobutane ligands in recognition of these lesions by DNA binding proteins and repair. The results demonstrate that distinctly different conformational and other physical properties of these crosslinks are responsible for their different affinity to HMG-box proteins and, consequently, for the different nucleotide excision repair of these lesions. Thus, the results suggest that because of the enantiomorphism of carrier amine ligands of cisplatin analogs, not only do their DNA crosslinks exhibit different conformational features, but also these adducts are processed differently by cellular components as a consequence of these conformational differences. As such, the results expand our knowledge of how stereochemistry of the carrier amine moiety in antitumor platinum(II) compounds influences some crucial processes underlying their toxicity toward cancer cells and provide a rational basis for the design of new platinum antitumor drugs and chemotherapeutic strategies.

Experimental Procedures

Starting Materials

Pt(*R,R*-DAB) and Pt(*S,S*-DAB) were prepared and characterized as described [22]. Cisplatin was synthesized and characterized in Lachema (Brno, Czech Republic). The stock solutions of the platinum complexes (0.5 mM in 10 mM $NaClO_4$) were prepared in the dark at 25°C. The synthetic oligodeoxyribonucleotides (Table 1) were purchased from IDT (Coralville, IA) and purified as described previously [61, 62]; in the present work their molar concentrations are related to the whole duplexes. Acrylamide, bis(acrylamide), and NaCN were from Merck Kga (Darmstadt, Germany). T4 DNA ligase and T4 polynucleotide kinase were purchased from New England Biolabs (Beverly, MA). [γ-^{32}P]ATP was from Amersham (Arlington heights, IL).

Preparation of Oligonucleotide Probes Containing Single, Site-Specific 1,2-GG Intrastrand CLs

The 20 bp oligonucleotide duplex TGGT/ACCA(1) (for its nucleotide sequence, see Table 1) uniquely and site-specifically intrastrand crosslinked by Pt(*R,R*-DAB), Pt(*S,S*-DAB), or cisplatin were prepared and characterized as described previously [32, 61, 63, 64]. The 20-mer oligonucleotides containing a single, site-specific 1,2-GG intrastrand CL of Pt(*R,R*-DAB), Pt(*S,S*-DAB), or cisplatin at the central TGGT sequence were used for preparation of linear 148 bp duplexes with a centrally located 1,2-GG intrastrand CL at nucleotides 75 and 76. Uniquely modified 20-mers were end-labeled to introduce a radiolabel at the tenth phosphodiester bond 5′ to the CL, annealed with a set of five complementary and partially overlapping oligonucleotides [ACCA(2), 64-mer(1), 64-mer(2), 61-mer, and 59-mer; for the nucleotide sequences, see Table 1], and ligated with T4 DNA ligase. Full-length substrates were separated from unligated products in a 6% denaturing polyacrylamide gel, purified by electroelution, reannealed, and stored in annealing buffer (50 mM Tris-HCl [pH 7.9], 100 mM NaCl, 10 mM $MgCl_2$, and 1 mM dithiothreitol) at −20°C. Other details of the purification of DNA substrates for NER were the same as described previously [65, 66].

Proteins

Expression and purification of recombinant rat full-length HMGB1 protein and its domains A (residues 1–84) and B (residues 85–180) were carried out as described [67, 68]. The residue numbering corresponds to that in the full-length HMGB1 protein (GenBank, accession number Y00463).

Electrophoretic Mobility Shift Assays with HMGB1 Domain Proteins

Radioactively labeled 20 bp probe DNAs (10 nM) were titrated with HMGB1a or HMGB1b proteins in 10 μl sample volumes in buffer I composed of 10 mM HEPES (pH 7.5), 10 mM $MgCl_2$, 50 mM LiCl, 100 mM NaCl, 1 mM spermidine, 0.2 mg/ml bovine serum albumin, and 0.05% v/v Nonidet P40. For all gel mobility shift experiments, samples were incubated on ice for 1 hr and made 7% in sucrose and 0.017% in xylene cyanol prior to being loaded on running, precooloed (4°C), prerun (300 V, 1–2 hr) 5% native polyacrylamide gels (29:1 acrylamide:bisacrylamide, 0.5× TBE buffer (45 mM Tris-HCl,

45 mM boric acid, and 1 mM EDTA [pH 8.3]). Gels were electrophoresed at 4°C and 300 V for ~1.5 hr, dried, exposed to a molecular-imaging plate, and analyzed on a Molecular dynamics phosphor imager (Storm 860 system), and the bands were quantitated with the ImageQuant software. For competition assays, unlabeled competitor 20-bp oligonucleotide probe [TGGT/ACCA(1) duplex] containing a single 1,2-GG intrastrand CL of Pt(*R,R*-DAB), Pt(*S,S*-DAB), or cisplatin (0–10 μM) was titrated against a radiolabeled 20 bp oligonucleotide probe [TGGT/ACCA(1) duplex] containing a single 1,2-GG intrastrand CL of cisplatin (10 nM) complexed with HMGB1a. Both oligonucleotides were mixed prior to addition of the protein at a concentration which afforded 60%–70% binding of the labeled probe in the absence of competitor [41, 69]. Apparent dissociation constants, $K_{D(app)}$, were estimated in the manner described in [40]. Each $K_{D(app)}$ is the average of at least two measurements.

Nucleotide Excision Assay

Oligonucleotide excision reactions were performed in CFEs prepared from the HeLa S3 and CHO AA8 cell lines as described [37, 70]. These extracts were kindly provided by J.T. Reardon and A. Sancar from the University of North Carolina (Chapel Hill, USA). In vitro repair of 1,2-GG intrastrand CLs of Pt(*R,R*-DAB) and Pt(*S,S*-DAB) was measured via an excision assay with these CFEs and 148 bp linear DNA substrates (vide supra) in the way described previously [37] with small modifications. The reaction mixtures (25 μl) contained 10 fmol of radiolabeled DNA, 50 μg of CFE, 20 μM dATP, dCTP, dGTP, and TTP in the reaction buffer (23 mM HEPES [pH7.9], 44 mM KCl, 4.8 mM $MgCl_2$, 0.16 mM EDTA, 0.52 mM dithiothreitol, 1.5 mM ATP, 5 μg of bovine serum albumin, and 2.5% glycerol) and were incubated at 30°C for 40 min. The 40 min time point was chosen for this study because it was in the linear part of the kinetics of removal of the 1,2-GG intrastrand CLs of the platinum compounds tested in the present work. For the protein inhibition studies, the full-length HMGB1 protein was added in 5 μl of dilution buffer (50 mM Tris-HCl [pH 7.5], 0.2 M NaCl, 1 mM EDTA, and 1 mM 2-mercaptoethanol), and the reaction mixtures were incubated on ice for 1 hr prior to the addition of CFE. DNA was deproteinized and precipitated by ethanol. The excision products were separated on 10% denaturing polyacrylamide gels and visualized with the Molecular Dynamics phosphor imager (Storm 860 system), and the bands were quantitated with ImageQuant software. With small modifications, mapping incision sites was performed as described in the preceding report [37]. In brief, the major excision product (gel purified) was further incubated for 10 min at 30°C with T4 DNA polymerase (0.25 units) in 10 μl of buffer composed of 50 mM Tris-HCl (pH 8.8), 15 mM $(NH_4)_2SO_4$, 7 mM $MgCl_2$, 0.1 mM EDTA, 50 mM β-mercaptoethanol, and 20 μg of BSA/ml, supplemented with 0.5 μg SmaI-digested pBluescript DNA and visualized by autoradiography after resolution in 10% denaturing polyacrylamide gel. Similar analyses with radiolabeled, platinated 20-mers (used in the nucleotide excision assays) were also used to identify the nucleotide(s) at which the exonuclease activity of T4 DNA polymerase is blocked 3′ to the lesion. The location of the 5′ incision site made by the excinuclease was determined by comparison with the length of excision products observed in the absence of T4 DNA polymerase digestion.

Acknowledgments

This research was supported by the Grant Agency of the Czech Republic (grant number 305/02/1552), the Grant Agency of the Academy of Sciences of the Czech Republic (grant number A5004101), and the Internal Grant Agency of the Ministry of Health of the Czech Republic (grant number NL6058-3/2000). The research of J.K. and V.B. was supported in part by an International Research Scholar's award from the Howard Hughes Medical Institute and by the Wellcome Trust (United Kingdom).

Received: January 11, 2002
Revised: February 12, 2002
Accepted: February 20, 2002

References

1. Sundquist, W.I., and Lippard, S.J. (1990). The coordination chemistry of platinum anticancer drugs and related compounds with DNA. Coord. Chem. Rev. *100*, 293–322.
2. Johnson, N.P., Butour, J.-L., Villani, G., Wimmer, F.L., Defais, M., Pierson, V., and Brabec, V. (1989). Metal antitumor compounds: the mechanism of action of platinum complexes. Prog. Clin. Biochem. Med. *10*, 1–24.
3. Sherman, S.E., Gibson, D., Wang, A.H.J., and Lippard, S.J. (1988). Crystal and molecular structure of *cis*-[$Pt(NH_3)_2${d(pGpG)}], the principal adduct formed by *cis*-diamminedichloroplatinum(II) with DNA. J. Am. Chem. Soc. *110*, 7368–7380.
4. Xu, Y.H., Natile, G., Intini, F.P., and Marzilli, L.G. (1990). Stereochemically controlled ligands influence atropisomerization of Pt(II) nucleotide complexes — evidence for head-to-head and stable a head-to-tail atropisomers. J. Am. Chem. Soc. *112*, 8177–8179.
5. Kiser, D., Intini, F.P., Xu, Y.H., Natile, G., and Marzilli, L.G. (1994). Atropisomerization of cis-bis(5′GMP)-platinum(II)-diamine complexes with non-C2-symmetrical asymmetric diamine ligands containing NH groups directed to one side of the coordination plane. Inorg. Chem. *33*, 4149–4158.
6. Guo, Z.J., Sadler, P.J., and Zang, E. (1997). Recognition of platinum(II) amine complexes by nucleotides: role of phosphate and carbonyl groups in ([N-15(3)]diethylenetriamine)-(guanosine 5′-monophosphate). Chem. Commun., 27–28.
7. Fouts, C.S., Marzilli, L.G., Byrd, R.A., Summers, M.F., Zon, G., and Shinozuka, K. (1988). HMQC and H-1 and P-31 NMR-studies of platinum amine adducts of tetradeoxyribonucleotides: relationship between P-31 shift and potential hydrogen-bonding interactions in pGpG moieties cross-linked by platinum. Inorg. Chem. *27*, 366–376.
8. Berners-Price, S.J., Frenkiel, T.A., Ranford, J.D., and Sadler, P.J. (1992). Nuclear magnetic resonance studies of N-H bonds in platinum anticancer complexes: detection of reaction intermediates and hydrogen bonding in guanosine 5′-monophosphate adducts of *cis*-[$PtCl_2(NH_3)_2$]. J. Chem. Soc. Dalton Trans., 2137–2139.
9. Bloemink, M.J., Heetebrij, R.J., Inagaki, K., Kidani, Y., and Reedijk, J. (1992). Reaction of unsymmetrically substituted derivatives of cisplatin with short oligodeoxynucleotides containing a -GpG- sequence: H-bonding interactions in pGpG moieties cross-linked by an asymmetric platinum complex enhancing the formation of one geometrical isomer. Inorg. Chem. *31*, 4656–4661.
10. Reedijk, J. (1992). The relevance of hydrogen bonding in the mechanism of action of platinum antitumor compounds. Inorg. Chim. Acta *198*, 873–881.
11. Berners-Price, S.J., Frey, U., Ranford, J.D., and Sadler, P. (1993). Stereospecific hydrogen-bonding in mononucleotide adducts of platinum anticancer complexes in aqueous-solution. J. Am. Chem. Soc. *115*, 8649–8659.
12. Berners-Price, S.J., Ranford, J.D., and Sadler, P.J. (1994). [H-1, N-15] NMR investigations of Pt-NH hydrogen-bonding in d(GpG), d(pGpG), and d(TpGpG)-N7,N7 adducts of $[Pt(en)]^{2+}$ in aqueous-solution. Inorg. Chem. *33*, 5842–5846.
13. Kidani, Y., Inagagi, K., and Tsukagoshi, S. (1976). Examination of antitumor activities of platinum complexes of 1,2-diaminocyclohexane isomers and their related complexes. Gann *67*, 921–922.
14. Kidani, Y., Inagaki, K., Iigo, M., Hoshi, A., and Kuretani, K. (1978). Antitumor activity of 1,2-diamminocyclohexane-platinum complexes against Sarcoma 180 ascites form. J. Med. Chem. *21*, 1315–1318.
15. Kidani, Y., Noji, M., and Tashiro, T. (1980). Antitumor activity of platinum(II) complexes of 1,2-diaminocyclohexane isomers. Gann *71*, 637–643.
16. Boudny, V., Vrana, O., Gaucheron, F., Kleinwächter, V., Leng, M., and Brabec, V. (1992). Biophysical analysis of DNA modified by 1,2-diaminocyclohexane platinum(II) complexes. Nucleic Acids Res. *20*, 267–272.
17. Noji, M., Okamoto, K., Kidani, Y., and Tashiro, T. (1981). Relation of conformation to antitumor activity of platinum(II) complexes

of 1,2-cyclohexanediamine and 2-(aminomethyl)cyclohexylamine isomers against leukemia P388. J. Med. Chem. *24*, 508–514.

18. Okamoto, K., Noji, M., Tashiro, T., and Kidani, Y. (1981). Preparation of platinum(II) complexes of diamine isomers [PtX(1,3-diamine)] (X = Cl_2, SO_4, $(NO_3)_2$, oxalato, D-glucuronato and D-gluconato) and determination of their antitumor activity against leukemia L1210. Chem. Pharm. Bull. *29*, 929–939.
19. Noji, M., Motoyama, S., Tashiro, T., and Kidani, Y. (1983). Synthesis and antitumor activity of Pt(II) complexes containing 2,3-diaminopropanol isomers. Chem. Pharm. Bull. (Tokyo) *31*, 1469–1473.
20. Noji, M., Gohchi, Y., and Kidani, Y. (1984). Preparation of antitumour platinum(II) complexes of 1,2-diphenylethylenediamine isomers and their interactions with DNA and its purine moieties. Chem. Biol. Interact. *51*, 37–48.
21. Coluccia, M., Correale, M., Giordano, D., Mariggio, M.A., Moscelli, S., Fanizzi, F.P., Natile, G., and Maresca, L. (1986). Mutagenic activity of some platinum complexes with monodentate and bidentate amines. Inorg. Chim. Acta *123*, 225–229.
22. Fanizzi, F.P., Intini, F.P., Maresca, L., Natile, G., Quaranata, R., Coluccia, M., Di Bari, L., Giordano, D., and Mariggio, M.A. (1987). Biological activity of platinum complexes containing chiral centers on the nitrogen or carbon atoms of a chelate diamine ring. Inorg. Chim. Acta *137*, 45–51.
23. Giannini, G., and Natile, G. (1991). Steric constraints inside the metal-coordination sphere as revealed by diastereotopic splitting of methylene protons. Inorg. Chem. *30*, 2853–2855.
24. Coluccia, M., Fanizzi, F.P., Giannini, G., Giordano, D., Intini, F.P., Lacidogna, G., Loseto, F., Mariggio, M.A., Nassi, A., and Natile, G. (1991). Synthesis, mutagenicity, binding to pBR-322 DNA and antitumor-activity of platinum(II) complexes with ethambutol. Anticancer Res. *11*, 281–287.
25. Vickery, K., Bonin, A.M., Fenton, R.R., O'Mara, S., Russell, P.J., Webster, L.K., and Hambley, T.W.J. (1993). Preparation, characterization, cytotoxicity, and mutagenicity of a pair of enantiomeric platinum(II) complexes with the potential to bind enantioselectively to DNA. J. Med. Chem. *36*, 3663–3668.
26. Fenton, R.R., Easdale, W.J., Er, H.M., O'Mara, S.M., McKeage, M.J., Russell, P.J., and Hambley, T.W. (1997). Preparation, DNA binding, and in vitro cytotoxicity of a pair of enantiomeric platinum(II) complexes, [(R)- and (S)-3-aminohexahydroazepine]-dichloro-platinum(II). Crystal structure of the S enantiomer. J. Med. Chem. *40*, 1090–1098.
27. Misset, J.L. (1998). Oxaliplatin in practice. Brit. J. Cancer Suppl. 4 *77*, 4–7.
28. Spingler, B., Whittington, D.A., and Lippard, S.J. (2001). 1,2-d(GpG) intrastrand cross-link formed by oxaliplatin with duplex DNA: a crystallographic study. J. Inorg. Biochem. *86*, 440.
29. Spingler, B., Whittington, D.A., and Lippard, S.J. (2001). 2.4 Å crystal structure of an oxaliplatin 1,2-d(GpG) intrastrand cross-link in a DNA dodecamer duplex. Inorg. Chem. *40*, 5596–5602.
30. Wei, M., Cohen, S.M., Silverman, A.P., and Lippard, S.J. (2001). Effects of spectator ligands on the specific recognition of intrastrand platinum-DNA cross-links by high mobility group box and TATA-binding proteins. J. Biol. Chem. *276*, 38774–38780.
31. Malina, J., Hofr, C., Maresca, L., Natile, G., and Brabec, V. (2000). DNA interactions of antitumor cisplatin analogs containing enantiomeric amine ligands. Biophys. J. *78*, 2008–2021.
32. Kasparkova, J., and Brabec, V. (1995). Recognition of DNA interstrand cross-links of cis-diamminedichloroplatinum(II) and its trans isomer by DNA-binding proteins. Biochemistry *34*, 12379–12387.
33. Zlatanova, J., Yaneva, J., and Leuba, S.H. (1998). Proteins that specifically recognize cisplatin-damaged DNA: a clue to anticancer activity of cisplatin. FASEB J. *12*, 791–799.
34. Ohndorf, U.M., Rould, M.A., He, Q., Pabo, C.O., and Lippard, S.J. (1999). Basis for recognition of cisplatin-modified DNA by high-mobility-group proteins. Nature *399*, 708–712.
35. Zamble, D.B., and Lippard, S.J. (1999). The response of cellular proteins to cisplatin-damaged DNA. In Cisplatin. Chemistry and Biochemistry of a Leading Anticancer Drug, B. Lippert, ed. (Zürich, Switzerland: Verlag Helvetica Chimica Acta; Weinheim, Germany: WILEY-VCH), pp. 73–110.
36. Zamble, D.B., Mu, D., Reardon, J.T., Sancar, A., and Lippard, S.J. (1996). Repair of cisplatin-DNA adducts by the mammalian excision nuclease. Biochemistry *35*, 10004–10013.
37. Reardon, J.T., Vaisman, A., Chaney, S.G., and Sancar, A. (1999). Efficient nucleotide excision repair of cisplatin, oxaliplatin, and bis-aceto-ammine-dichloro-cyclohexylamine-platinum(IV) (JM216) platinum intrastrand DNA diadducts. Cancer Res. *59*, 3968–3971.
38. Moggs, J.G., Szymkowski, D.E., Yamada, M., Karran, P., and Wood, R.D. (1997). Differential human nucleotide excision repair of paired and mispaired cisplatin-DNA adducts. Nucleic Acids Res. *25*, 480–490.
39. Cohen, S.M., Mikata, Y., He, Q., and Lippard, S.J. (2000). HMG-domain protein recognition of cisplatin 1,2-intrastrand d(GpG) cross-links in purine-rich sequence contexts. Biochemistry *39*, 11771–11776.
40. He, Q., Ohndorf, U.-M., and Lippard, S.J. (2000). Intercalating residues determine the mode of HMG1 domains A and B binding to cisplatin-modified DNA. Biochemistry *39*, 14426–14435.
41. Dunham, S.U., and Lippard, S.J. (1997). DNA sequence context and protein composition modulate HMG-domain protein recognition of ciplatin-modified DNA. Biochemistry *36*, 11428–11436.
42. Sancar, A. (1996). DNA excision repair. Ann. Rev. Biochem. *65*, 43–81.
43. Wood, R.D. (1996). DNA repair in eukaryotes. Ann. Rev. Biochem. *65*, 135–167.
44. Naegeli, H. (1997). Mechanisms of DNA Damage Recognition in Mammalian Cells (New York: Springer).
45. Reardon, J.T., and Sancar, A. (1998). Molecular mechanism of nucleotide excision repair in mammalian cells. In Advances in DNA Damage and Repair, M. Dizdaroglu and A. Karakaya, eds. (New York: Plenum Publishing Corp.), pp. 377–393.
46. Jones, S.L., Hickson, I.D., Harris, A.L., and Harnett, P.R. (1994). Repair of cisplatin-DNA adducts by protein extracts from human ovarian carcinoma. Int. J. Cancer *59*, 388–393.
47. Koberle, B., Masters, J.R.W., Hartley, J.A., and Wood, R.D. (1999). Defective repair of cisplatin-induced DNA damage caused by reduced XPA protein in testicular germ cell tumours. Curr. Biol. *9*, 273–276.
48. Li, M.J., and Yang, L.Y. (1999). Use of novel plasmid constructs to demonstrate fludarabine triphosphate inhibition of nucleotide excision repair of a site-specific 1,2-d(GpG) intrastrand cisplatin adduct. Int. J. Oncol. *15*, 1177–1183.
49. Huang, J.-C., Svoboda, D.L., Reardon, J.T., and Sancar, A. (1992). Human nucleotide excision nuclease removes thymine dimers from DNA by incising the 22nd phosphodiester bond 5′ and the 6th phosphodiester bond 3′ to the photodimer. Proc. Natl. Acad. Sci. USA *89*, 3664–3668.
50. Huang, J.C., Zamble, D.B., Reardon, J.T., Lippard, S.J., and Sancar, A. (1994). HMG-domain proteins specifically inhibit the repair of the major DNA adduct of the anticancer drug cisplatin by human excision nuclease. Proc. Natl. Acad. Sci. USA *91*, 10394–10398.
51. Jamieson, E.R., and Lippard, S.J. (1999). Structure, recognition, and processing of cisplatin-DNA adducts. Chem. Rev. *99*, 2467–2498.
52. Grabner, S., Plavec, J., Bukovec, N., Di Leo, D., Cini, R., and Natile, G. (1998). Synthesis and structural characterization of platinum(II)-acyclovir complexes. J. Chem. Soc. Dalton Trans., 1447–1451.
53. Cini, R., Grabner, S., Bukovec, N., Cerasino, L., and Natile, G. (2000). Synthesis and structural characterisation of a new form of bis(acyclovir)(ethylenediamine)platinum(II): correlation between the puckering of the carrier ligand and the canting of the nucleobases. Eur. J. Inorg. Chem., 1601–1607.
54. Pilch, D.S., Dunham, S.U., Jamieson, E.R., Lippard, S.J., and Breslauer, K.J. (2000). DNA sequence context modulates the impact of a cisplatin 1,2-d(GpG) intrastrand cross-link and the conformational and thermodynamic properties of duplex DNA. J. Mol. Biol. *296*, 803–812.
55. Hofr, C., Farrell, N., and Brabec, V. (2001). Thermodynamic properties of duplex DNA containing a site-specific d(GpG) intrastrand crosslink formed by an antitumor dinuclear platinum complex. Nucleic Acids Res. *29*, 2034–2040.

56. McA'Nulty, M., and Lippard, S.J. (1995). Consequences of HMG-domain binding to cisplatin-modified DNA. Nucleic Acids Mol. Biol. *9*, 264–284.
57. Whitehead, J.P., and Lippard, S.J. (1996). Proteins that bind to and mediate the biological activity of platinum anticancer drug-DNA adducts. In Metal Ions in Biological Systems, Vol. *32*, A. Sigel and H. Sigel, eds. (New York: Marcel Dekker), pp. 687–726.
58. He, Q., Liang, C.H., and Lippard, S.J. (2000). Steroid hormones induce HMG1 overexpression and sensitize breast cancer cells to cisplatin and carboplatin. Proc. Natl. Acad. Sci. USA *97*, 5768–5772.
59. Hoffmann, J.S., Locker, D., Viliani, G., and Leng, M. (1997). HMG1 protein inhibits the translesion synthesis of the major DNA cisplatin adduct by cell extracts. J. Mol. Biol. *270*, 539–543.
60. Vaisman, A., Lim, S.E., Patrick, S.M., Copeland, W.C., Hinkle, D.C., Turchi, J.J., and Chaney, S.G. (1999). Effect of DNA polymerases and high mobility group protein 1 on the carrier ligand specificity for translesion synthesis past platinum-DNA adducts. Biochemistry *38*, 11026–11039.
61. Brabec, V., Reedijk, J., and Leng, M. (1992). Sequence-dependent distortions induced in DNA by monofunctional platinum(II) binding. Biochemistry *31*, 12397–12402.
62. Kasparkova, J., Farrell, N., and Brabec, V. (2000). Sequence specificity, conformation, and recognition by HMG1 protein of major DNA interstrand cross-links of antitumor dinuclear platinum complexes. J. Biol. Chem. *275*, 15789–15798.
63. Bellon, S.F., Coleman, J.H., and Lippard, S.J. (1991). DNA unwinding produced by site-specific intrastrand cross-links of the antitumor drug *cis*-diamminedichloroplatinum(II). Biochemistry *30*, 8026–8035.
64. Brabec, V., Neplechova, K., Kasparkova, J., and Farrell, N. (2000). Steric control of DNA interstrand cross-link sites of trans platinum complexes: specificity can be dictated by planar non-leaving groups. J. Biol. Inorg. Chem. *5*, 364–368.
65. Matsunaga, T., Mu, D., Park, C.-H., Reardon, J.T., and Sancar, A. (1995). Human DNA repair excision nuclease. J. Biol. Chem. *270*, 20862–20869.
66. Buschta-Hedayat, N., Buterin, T., Hess, M.T., Missura, M., and Naegeli, H. (1999). Recognition of nonhybridizing base pairs during nucleotide excision repair of DNA. Proc. Natl. Acad. Sci. USA *96*, 6090–6095.
67. Stros, M., and Muselikova, E. (2000). A role of basic residues and the putative intercalating phenylalanine of the HMG-1 box B in DNA supercoiling and binding to four-way DNA junctions. J. Biol. Chem. *275*, 35699–35707.
68. Stros, M. (1998). DNA bending by the chromosomal protein HMG1 and its high mobility group box domains. Effect of flanking sequences. J. Biol. Chem. *273*, 10355–10361.
69. Long, K.S., and Crothers, D.M. (1995). Interaction of human-immunodeficiency-virus type-1 Tat-derived peptides with TAR RNA. Biochemistry *34*, 8885–8895.
70. Manley, J.L., Fire, A., Cano, A., Sharp, P.A., and Gefter, M.L. (1980). DNA-dependent transcription of adenovirus genes in a soluble whole-cell extract. Proc. Natl. Acad. Sci. USA *77*, 3855–3859.
71. Lohman, T.M., and Mascotti, D.P. (1992). Thermodynamics of ligand-nucleic acid interactions. Methods Enzymol. *212*, 400–424.
72. Lin, S.-Y., and Riggs, A.D. (1972). lac repressor binding to non-operator DNA: detailed studies and a comparison of equilibrium and rate competition methods. J. Mol. Biol. *72*, 671–690.