The results presented describe the effects of various spectator ligands, attached to a platinum 1,2-intrastrand d(GpG) cross-link in duplex DNA, on the binding of high mobility group box (HMGB) domains and the TATA-binding protein (TBP). In addition to cisplatin-modified DNA, 15-base pair DNA probes modified by \( \text{Pt(1R,2R-diaminocyclohexane)}^{2+} \), \( \text{cis-Pt(NH}_3\text{)(cyclohexylamine)}^{2+} \), \( \text{(Pt(ethylene-diamine)}^{2+} \), \( \text{cis-Pt(NH}_3\text{(cyclobutylamine)}^{2+} \), and \( \text{cis-Pt(NH}_3\text{(2-picoline)}^{2+} \) were examined. Electrophoretic mobility shift assays show that both the A and B domains of HMGB1 as well as TBP discriminate between different platinum-DNA adducts. HMGB1 domain A is the most sensitive to the nature of the spectator ligands on platinum. The effect of the spectator ligands on protein binding also depends highly on the base pairs flanking the platinumated d(GpG) site. Double-stranded oligonucleotides containing the AG*G*C sequence, where the asterisks denote the sites of platination, with different spectator ligands are only moderately discriminated by the HMGB proteins and TBP, but the recognition of dsTG*G*A is highly dependent on the ligands. The effects of HMGB1 overexpression in a BG-1 ovarian cancer cell line, induced by steroid hormones, on the sensitivity of cells treated with \( \text{Pt(1R,2R-diaminocyclohexane)}^{2+} \) and \( \text{cis-Pt(NH}_3\text{(cyclohexylamine)}^{2+} \) were also examined. The results suggest that HMGB1 protein levels influence the cellular processing of cis-\( \text{Pt(NH}_3\text{(cyclohexylamine)}^{2+} \) but not \( \text{Pt(1R,2R-diaminocyclohexane)}^{2+} \) DNA lesions. This result is consistent with the observed binding of HMGB1a to platinum-modified dsTG*G*A probes but not with the binding affinity of HMGB1a to platinum-damaged dsAG*G*C oligonucleotides. These experiments reinforce the importance of sequence context in platinum-DNA lesion recognition by cellular proteins.

\* This work was supported by Grant CA34992 from the National Cancer Institute. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

\( ^\text{S} \) The on-line version of this article (available at http://www.jbc.org) contains Tables S1 and S2 and Fig. S1.

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\( ^\text{1} \) The abbreviations used are: cisplatin, \( \text{cis-diaminedichloro(platinum(II)) \text{ (cisplatin)}} \); 1,2-intrastrand cross-links at the d(GpG) and d(ApG) sites.

\( ^\text{2} \) cis-DDP, \( \text{cis-diaminedichloro(platinum(II)) \text{ (cisplatin)}} \), one of the most effective anticancer drugs, is widely used to treat a variety of malignancies (1). The clinical efficacy of cisplatin is severely undermined, however, by dose-limiting side effects and the intrinsic or acquired resistance of some tumors (2). The cisplatin analog, \( \text{cis-diammine-1,1’-cyclobutanedicarboxylato(platinum(II)) \text{, or carboplatin, was introduced into the clinic because of its reduced toxicity relative to cisplatin, but it is usually ineffective against cisplatin-resistant tumors (3). Recently, new platinum compounds have emerged that demonstrate promising anticancer activities and a lack of cross-resistance with cisplatin (4). Several compounds are now in various stages of clinical testing and have exhibited impressive results for the treatment of certain tumors. Oxaliplatin (Fig. 1) has been approved in Europe, in combination with 5-fluorouracil, for the treatment of advanced colorectal cancer. \( \text{cis-trans-cis-Ammine(cyclohexylamine)diacetatodichloro(platinum(IV)) \text{ (JM216; Fig. 1), an orally active platinum(IV) compound, is currently in phase III clinical trials for prostate cancer (5). \text{cis-Amminedichloro(2-methylpyridine)platinum(II) \text{ (ZD0473 or AMD473; Fig. 1)} \text{ has recently entered phase III clinical trials for ovarian cancer (6).}} \)

Despite the large amount of clinical data, there are relatively few attempts to understand why these new platinum complexes might be effective. The cytotoxicity of platinum compounds derives from the formation of DNA adducts, the major ones being 1,2-intrastrand cross-links at the d(GpG) and d(ApG) sites (7). Cisplatin modification produces marked local distortions in the DNA duplex (8, 9) that are recognized by a number of cellular proteins (1). Specific interactions between cisplatin 1,2-intrastrand cross-links and cellular proteins modulate the sensitivity of tumor cells to the drug. In particular, protein-DNA interactions induced by cisplatin inhibit replication (10), shield the adducts from nucleotide excision repair (11) and interfere with transcription by recruiting transcription factors from their native binding sites (12, 13). Such interactions may also modulate the different cellular responses to platinum compounds with varying spectator ligands, ligands that remain bound to platinum after formation of Pt-DNA adducts. As with cisplatin-DNA damage, the 1,2-intrastrand d(GpG) and d(ApG) cross-links formed by these closely related platinum compounds most likely contribute to their cytotoxicity. For example, oxaliplatin forms mainly 1,2-intrastrand d(GpG) cross-links both in vitro and in cells (14, 15). Therefore, understanding how these spectator ligands, ammonia in the case of cisplatin and 1,2-diaminocyclohexane in the case of oxaliplatin, affect the binding of cellular proteins to platinumated DNA may help to distinguish the manner by which the proc-
essing of their lesions in the cell can differ from that of cisplatin-damaged DNA.

HMGB1 and TBP are abundant nuclear proteins that bind tightly to cisplatin-modified DNA, although they share no sequence or structural homology (13, 16). HMGB1 is a chromosomal protein that functions in a number of biological processes, probably by modulating DNA architecture and facilitating formation of higher order nucleoprotein complexes (17). TBP is one member of the transcription factor IID protein assembly involved in eukaryotic transcription initiation (18). Both proteins have been implicated in the biological activity of cisplatin. Cisplatin-modified DNA can divert TBP from its natural binding site, the TATA box, thus inhibiting transcription initiation (13). When binding to platinum-DNA adducts, HMGB1 can interfere with repair of the damaged DNA (11, 19). HMGB1 contains two HMG box domains (HMGB1a and HMGB1b), 80-amino acid DNA-binding motifs that recognize bent DNA structures (20, 21). The binding interactions between HMG box proteins and cisplatin-modified DNA were revealed by an x-ray crystal structure determination of HMGB1a in complex with a 16-bp oligonucleotide adducts. TBP can bind cisplatin-DNA adducts with different ligands.

**Experimental Procedures**

**Platinum Complexes**—Cisplatin, [Pt(dach)Cl2], and [Pt(en)Cl2] are prepared using standard protocols (26). The mixed ligand complexes cis-[Pt(NH3)2(NH2Cy)Cl2], cis-[Pt(NH3)2(NH2Cba)Cl2], and cis-[Pt(NH3)2(2-pic)I2] were obtained as described in the literature (27, 28).

**Proteins**—HMGB1a and HMGB1b proteins were prepared by E. R. Jameson (29, 30), and the C-terminal DNA binding domain of the yeast TATA-binding protein (yTBP) was prepared as described previously (31).

**Oligonucleotide Probes**—Oligonucleotide probes, top strand 5′-GGAGAGTCCGAGAAG-3′ (tsAGGC) and 5′-GGCTCTCAGGCTCTTC-3′ (tsGGAG), were prepared (1.0-μmol scale) by using standard phosphoramidite methods on an Applied Biosystems 392 DNA/RNA synthesizer. All phosphoramidites were obtained from Glen Research. After automated synthesis, the oligonucleotides were depurinated with ammonium hydroxide by incubating the crude reaction mixtures at 65 °C for 1–2 h. The probes were purified on 12% denaturing polyacrylamide gels (7.5 M urea, 19:1 acrylamide:bisacrylamide ratio, 90 mM Tris borate, and 1.0 mM EDTA, pH 8.3) run at constant voltage (300 V) with ambient temperature water cooling. Analysis by ion exchange HPLC showed a single peak for all oligonucleotides.

Platinum compounds were activated by mixing 2 equivalents of silver nitrate with 1 equivalent of the chloride or iodide platinum complex in 1–5 ml of water for 6–22 h. The mixtures were protected from light and centrifuged to remove precipitated silver chloride or silver iodide. Oligonucleotides with isolated d(GpG) sites (tsAGGC and tsGGAG) were activated with 2.0–2.2 equivalent of the activated platinum complex in a solution containing 10 mM sodium phosphate (pH 6.8) at 37 °C for 6–22 h. All platinated oligonucleotides were purified by ion exchange HPLC ( Dionex NucleoPac PA-100, 9 × 250 mm, 10% acetonitrile, 25 mM ammonium acetate, linear gradient from 0.25 to 0.5 M NaCl over 30 min). The purified probes were diazylated against water by using Slide-A-Lyzer cassettes (3500 molecular weight cut-off, Pierce). All platinated oligonucleotides products were confirmed by electrospray ionization or matrix-assisted laser-desorption time-of-flight mass spectrometry.

The oligonucleotides 5′-GGAGAGTCGGAGAGG-3′ (bsAGGC, complement to tsAGGC) and 5′-GGAGAGTCGGAGAGG-3′ (bsGGAG, complement to tsGGAG) were radiolabeled in a solution (20–50 μl) containing

2 Supporting information is available. A table of mass spectra for platinated oligonucleotides is available free of charge via the Internet at pubs.acs.org.
ing 70 mM Tris-HCl (pH 7.6), 10 mM MgCl₂, 5 mM dithiothreitol, and 20 μCi of γ³²P-labeled ATP (PerkinElmer Life Sciences). The oligonucleotide solutions were mixed with 10–20 units of T4 polynucleotide kinase (New England Biolabs) and incubated at 37 °C. After 45 min, an additional 10–20 units of T4 polynucleotide kinase were added, and the mixtures were incubated for an additional 45 min at 37 °C. The reaction mixtures were spun dialyzed through G-25 Sephadex Quickspin columns (Roche Molecular Biochemicals). The effluents were diluted with water to a total volume of 100 μl and then extracted with 2 × 100 μl of a 25:24:1 phenol:CHCl₃/isooamyl alcohol mixture. Finally, the DNA was isolated by ethanol precipitation.

The platinated top strand oligonucleotides were combined in 5–10-fold excess with the corresponding γ³²P-radioabeled bottom strand in a solution containing 10 mM Tris, 10 mM MgCl₂, and 50 mM NaCl. The strands were annealed by heating to 45 °C for 15 min, followed by cooling to 4 °C over several hours. The double-stranded products were stored at −20 °C.

Electrophoretic Mobility Shift Assays (EMSA)–EMSA for HMGB1a and HMGB1b were performed as described previously (32, 33). Protein (30 nm for HMGB1a or 340 nm for HMGB1b) and DNA probe (12–17 nm, 5000–10000 cpm/μl) were incubated on ice for 30 min in a solution containing 10 mM Hepes (pH 7.5), 10 mM MgCl₂, 50 mM LiCl, 100 mM NaCl, 1 mM spermidine, 0.2 mg/ml bovine serum albumin, and 0.05% Nonidet P-40 (15–μl reaction mixtures). The reaction mixtures were loaded onto 10% native polyacrylamide gels prepared with 0.5 × Tris-borate-EDTA ( prerun for at least 1 h at 300 V and 4 °C). The gels were run at 300 V and 4 °C for 1.5 h. The gels were dried under vacuum at 80 °C and exposed to a molecular imaging plate overnight.

The images were recorded by using a Bio-Rad GS-525 Molecular Imager. The relative amounts of DNA-protein complex and total DNA were obtained by integrating the volumes of their respective bands (total DNA) by using the Multi-analyst software package (Bio-Rad). The theta value (θ) was calculated according to the following equation.

\[ \theta = \frac{\text{protein-DNA complex/total DNA}}{\text{Eq. 1}} \]

EMSA for γTBPc were performed as reported (31). γTBPc (22.5 nM) and probe DNA (15–17 nm, 5000–10000 cpm/μl) were mixed in a 12-μl solution containing 25 mg/μl poly(dGdC) competitor, 20 nm KCl, 5.0 mM MgCl₂, 10 mM dithiothreitol, 0.2 mg/ml bovine serum albumin, and 10% glycerol and were incubated at 30 °C for 15 min, followed by cooling to 4 °C for 15 min. The reaction mixtures were then stained with a 1% methylene blue, 50% ethanol solution and counted. Kill curves were obtained by plotting the percentage of surviving cells against the concentration of the platinum compound.

RESULTS

Synthesis of Platinum-modified DNA Probes—The 15-bp oligonucleotide probes were synthesized and purified to afford pure materials as determined by analytical ion exchange HPLC. Platinum complexes were activated by reaction with 2 equivalents of silver nitrate in the dark. Reactions of tsTGGA with cis-[Pt(NH₃)₂(OH₂)₂]²⁺, [Pt(dach)(OH₂)₂]²⁺, and [Pt(en) (OH₂)²⁺] gave each one major product, which was purified by ion exchange HPLC. Reactions of tsTGGA with cis-[Pt(NH₃)₃(NH₂Cy)(OH₂)²⁻], cis-[Pt(NH₃)₂(NH₂Cy)(OH₂)²⁻], and cis-[Pt(NH₃)₂(2-pic)(OH₂)²⁺] gave two products with very similar retention times. The two products for each of these reactions were presumed to be the 5’ and 3’ orientation isomers of the platinum-DNA complex (24) and were purified together by ion exchange HPLC. All reactions of the tsAGGC probes with activated platinum complexes gave single products as revealed by ion exchange HPLC. For the tsAGGC probes modified with mixed ammine platinum complexes, it is possible that two orientational isomers were formed, but they could not be resolved by ion exchange HPLC. Alternatively, one orientational isomer might be formed exclusively in these reactions, perhaps because of an influence on the platination reaction by the bases flanking the GG site. All probes were checked by platinum atomic absorption spectroscopy and mass spectrometry.

Binding Affinity of HMGB1a for Pt-DNA Adducts—The binding of HMGB1a to six Pt-DNA duplexes with dsAG²⁺G⁴C and dsTG²⁺G⁴A flanking sequences was examined by EMSA experiments (Fig. 2). Also, binding to an unplatinated control sequence, either dsAGGCG or dsTGAGA, was evaluated. As expected, unplatinated oligonucleotide probes showed no protein binding, and cisplatin-modified probes formed the most DNA-protein complexes at a fixed HMGB1a concentration of 30 nm.

The affinity of HMGB1a for the dsTG²⁺G⁴A probes varies as cisplatin > cis-[Pt(NH₃)₂(NH₂Cy)(OH₂)²⁺] ≈ cis-[Pt(NH₃)₂(2-pic)(OH₂)²⁺] ≈ cis-[Pt(NH₃)₂(NH₂Cy)(OH₂)²⁻] > [Pt(en)]²⁺ ≈ [Pt(dach)]²⁺. The ratio (θ) of protein-bound nucleotide to total nucleotide for
Effects of Spectator Ligands

DNA lesions when compared with HMGB1a. HMGB1b binds the dsAG*G*C flanking sequence better than dsTG*G*A, on all probes (32). There are fewer differences in binding affinity for dsAG*G*C than dsTG*G*A probes, further demonstrating the influence of the flanking sequence for protein binding. Like HMGB1a, binding of HMGB1b with (Pt(dach))2+-modified DNA is highly dependent on the flanking sequence; HMGB1b binds AG*G*C about 6-fold stronger than TG*G*A.

Binding Affinity of TBP for Pt-DNA Adducts—TBP binds to the platinum-DNA probes as tightly as HMGB1a.3 To investigate the specific interaction of TBP with platinated DNA, 12 μg/μl poly(dGdC) was included in the binding mixture to reduce nonspecific binding of TBP with linear DNA. As shown by the EMSA experiments (Fig. 4), TBP binds more strongly to dsAG*G*C than to dsTG*G*A. TBP does not discriminate well between the dsAG*G*C probes but does selectively bind the various dsTG*G*A probes. TBP binds to cisplatin-modified dsTG*G*A 2–20-fold more strongly than any of the other platinum-modified probes. Most notably, the affinity of TBP to the (Pt(dach))2+-modified dsTG*G*A probe is significantly diminished relative to cisplatin, which is consistent with the recognition of this probe by HMGB1a and HMGB1b.

The results of the EMSAs indicate that HMGB box domains and TBP discriminate between Pt-DNA adducts containing different spectator ligands; however, the distinction between lesions can vary greatly with flanking sequence. HMGB1a binds more selectively to different platinum-DNA adducts than HMGB1b or TBP.

Effects of Steroid Hormone on the Cytotoxicity of the Plati-
creased by cotreatment with 0.1 M estrogen and progesterone. As observed in previous studies (19), the sensitivity of BG-1 cells to cisplatin is increased by cotreatment with 0.1 µM estrogen and 0.1 µM progesterone (Fig. 5). The cytotoxicity curves revealed that cotreatment with hormones sensitized BG-1 cells to cis-[Pt(NH₃)₂(NH₂Cy)Cl₂] compared with those modified with cisplatin under identical binding conditions. This result is in good agreement with previous data obtained for HMGB1 (23). In contrast, the binding of HMGB1a to the dsAG*G*C probes is much less selective. This observation is consistent with the fact that HMGB1a has a higher binding affinity for platinum-DNA cross-links than HMGB1b (29). These results suggest that HMGB1a dominates the interaction between HMGB1 and platinum-damaged DNA.

**DISCUSSION**

**HMGB1a Mediates the Differential Recognition of Platinum-DNA Adducts by HMGB1**—Previous EMSA studies suggested that HMGB1 has reduced affinity, relative to cisplatin, for the DNA adducts formed by [Pt(dach)Cl₂] and cis-[Pt(NH₃)₂(NH₂Cy)Cl₂] (23). It is not known, however, whether the decreased binding of full-length HMGB1 is a consequence of reduced affinity for the individual HMGB1 domains and, if so, which of the two domains better mediates the differential recognition of various platinum-DNA adducts by HMGB1. To address these questions, we studied the binding of the individual HMGB box domains to DNA adducts formed by platinum compounds with various spectator ligands. In addition, because the interactions between HMGB1a and cisplatin-modified DNA have been characterized in considerable detail, studies of the binding of HMGB1a to different platinum-DNA adducts allow us to speculate how the spectator ligands might perturb the protein-DNA interaction.

The EMSA experiments presented here reveal that HMGB1a forms about half the amount of protein-DNA adducts with dsAG*G*C probes modified with [Pt(dach)Cl₂] or cis-[Pt(NH₃)₂(NH₂Cy)Cl₂] compared with those modified with cisplatin under identical binding conditions. This result is in good agreement with previous data obtained for HMGB1 (23). In contrast, the binding of HMGB1b to the dsAG*G*C probes is much less selective. This observation is consistent with the fact that HMGB1a has a higher binding affinity for platinum-DNA cross-links than HMGB1b (29). These results suggest that HMGB1a dominates the interaction between HMGB1 and platinum-damaged DNA.

**Ligand Effects on Protein Binding Are Flanking Sequence-dependent**—Our results demonstrate that HMG box proteins and TBP can distinguish between platinum adducts modified with different spectator ligands. Previous data revealed that the affinity of HMGB1 proteins for cisplatin-modified DNA depends on the base pairs immediately flanking the platinum adduct (32, 33). Such flanking sequence dependence is especially prominent in the case of HMGB1a, which has a binding affinity that varies by several orders of magnitude for cisplatin-DNA adducts with different flanking sequences. Therefore, it is important to examine trends in protein-DNA interactions in different sequence contexts. In this study, we investigated the binding of the HMGB box proteins to DNA probes with two flanking sequences, dsAG*G*C and dsTG*G*A. The results indicate that the dsAG*G*C probes with different spectator ligands are only moderately differentiated by HMGB1a and even less so by HMGB1b and TBP. On the other hand, the recognition of dsTG*G*A probes by both HMGB box proteins and TBP varies to a great degree depending on the spectator ligands.

The EMSAs indicate that HMGB1a has a similarly reduced affinity for the dsAG*G*C adducts of [Pt(dach)]²⁺ and cis-[Pt(NH₃)₂(NH₂Cy)]²⁺, compared with those of cisplatin and are consistent with published results for HMGB1 (23). When binding to dsTG*G*A adducts, however, HMGB1a exhibits much higher selectivity compared with the same platinum adducts in dsAG*G*C oligonucleotides. The affinity of HMGB1a for the [Pt(dach)]²⁺ adduct is greatly decreased, but its binding for cis-[Pt(NH₃)₂(NH₂Cy)]²⁺ is only modestly decreased. The stronger discrimination of the dsTG*G*A oligonucleotides by HMGB1a suggests that HMGB1a is more sensitive to differences in the flanking sequences of DNA adducts than is HMGB1b.

The EMSA experiments presented here reveal that HMGB1a forms about half the amount of protein-DNA adducts with dsAG*G*C probes modified with [Pt(dach)Cl₂] or cis-[Pt(NH₃)₂(NH₂Cy)Cl₂] compared with those modified with cisplatin under identical binding conditions. This result is in good agreement with previous data obtained for HMGB1 (23). In contrast, the binding of HMGB1b to the dsAG*G*C probes is much less selective. This observation is consistent with the fact that HMGB1a has a higher binding affinity for platinum-DNA cross-links than HMGB1b (29). These results suggest that HMGB1a dominates the interaction between HMGB1 and platinum-damaged DNA.
box proteins compared with the dsAG*G*C DNA probes may be a consequence of different binding modes of protein-DNA complexes. To understand the sequence dependence of HMG box proteins binding to platinum-damaged DNA, however, further biochemical and structural studies are required.

**Platinum-DNA Probes with the Dach Ligand Are Poorly Recognized**—Compared with the other spectator ligands examined in this study, the chelating 1,2-diaminocyclohexane ligand has the largest effect on the binding affinity of HMG box domains and TBP to the dsTG*G*A probes (Figs. 2–4). For the dsAG*G*C probes, even when the overall attenuation of binding across the ligand set is small, probes modified by the dach complex display considerably reduced binding to HMG box proteins and TBP. The x-ray structure of HMG1a bound to cisplatin-modified DNA shows protein binding in the minor groove. It is therefore unlikely that HMG1a interacts directly with the spectator ligands (22). However, the binding of HMG1a increases the bending of the DNA toward the major groove, where the spectator ligands reside. One possible explanation for the present observed ligand effect is that the bulky dach group hinders the additional DNA bending that is required to form the protein-DNA complex. Consistent with this hypothesis is the fact that lesions formed with less bulky ethylenediamine group and the more flexible nonchelating (ammine)(cyclobutylamine) and (ammine)(cyclohexylamine) complexes are generally more tightly bound by the proteins. The binding of the dsAG*G*C probes by HMG1b, however, is inconsistent with this trend, which may be a consequence of the very low binding selectivity of HMG1b for these probes.

Recently, the structure of a double-stranded DNA dodecamer containing the [Pt(dach)]^2+ and 1,2-d(GpG) intrastrand cross-link was determined by x-ray crystallography (35). The dihedral angle between the two guanine base planes is 25°, similar to that (28°) in the x-ray structure of the cisplatin analog (9). The overall structures of the [Pt(dach)]^2+ and cisplatin adducts are quite similar except for a hydrogen bond that forms between the pseudo-equatorial NH of the dach ligand and the O-6 atom of the 3'-G base. Comparison of these structures would not appear prima facie to support the above hypothesis that the dach ligand restricts the bend angle of the platinum-DNA cross-link. As indicated by the x-ray structure of HMG1a complexed with cisplatin-modified DNA (22), however, the protein induces further bending of the DNA and the formation of a much greater guanine-guanine dihedral angle (75°). It is possible that the dach group restricts the additional DNA bending required for HMG1a binding. Further study is warranted to elucidate whether the DNA bend angle, the hydrogen bond between the NH of the dach ligand and the O-6 atom of the 3'-G of the d(GpG) lesion observed in the [Pt(dach)]^2+-DNA adduct or some other feature results in the reduced interaction between the HMG box proteins and [Pt(dach)]^2+-DNA adducts.

**TBP Differentially Recognizes Platinum-DNA Adducts**—As mentioned in the previous section and shown in Fig. 4, TBP also binds selectively to platinum-DNA adducts having different spectator ligands. The ligand dependence of binding by TBP is similar to that observed for the HMG box proteins. This result suggests that common structural factors govern the ligand dependence of protein binding to the platinum-DNA adducts. Although HMG1a and TBP both bind cisplatin-modified DNA with high affinity (29), they share no sequence homology or structural similarity, suggesting that other platinum-DNA-binding proteins may similarly discriminate between adducts with different spectator ligands. This hypothesis remains to be tested by studies with other damage recognition proteins.

Our results reveal that the ligand dependence of binding to platinated DNA by TBP and HMG box proteins varies with flanking sequence in a similar manner. TBP discriminates well between the different adducts formed by platinum complexes on the dsTG*G*A but not the dsAG*G*C, probes. A previous study using a filter binding assay demonstrated that TBP binds similarly to DNA globally modified by cisplatin or [Pt(dach)]^2+ (36). The present results are consistent with this finding. TBP binds with higher affinity to dsAG*G*C than dsTG*G*A DNA. Thus, in globally platinated DNA, the former adduct would afford a greater population of TBP complexes, for which there is little discrimination between spectator ligands. TBP binding to cisplatin-DNA adducts has been investigated for substrate probes containing a much wider range of sequences flanking the lesion site and will be reported elsewhere.

**Interaction between HMGB1 and Platinum-DNA Adducts in Cells**—The cytotoxicity of cisplatin depends on the formation of DNA adducts, particularly 1,2-intrastrand d(GpG) and d(ApG) cross-links. There is good evidence that the level of the intrastrand adducts measured in cells of ovarian and testicular cancer patients correlates with the response to cisplatin treatment (34, 37, 38). Oxaliplatin and other related platinum complexes also form mainly the 1,2-intrastrand d(GpG) and d(ApG) cross-links, and they share a similar mode of action with cisplatin. Because the different cytotoxicity of these compounds may also derive, at least in part, from the processing of their DNA adducts (3), it is of interest to study the recognition of these adducts by cellular proteins.

To investigate how HMGB1 interacts with [Pt(dach)]^2+ and cis-[Pt(NH3)(NH2Cy)]^2+-modified DNA in cells, the effects of steroid hormones on the cytotoxicity of cisplatin, [Pt(dach)Cl2], and cis-[Pt(NH3)(NH2Cy)Cl] were examined. Previous studies indicate that cells with steroid hormone receptors, such as the ovarian cancer BG-1 and breast cancer MCF-7 cell lines, overexpress HMGB1 protein in response to hormone treatment (19). The roughly 2-fold increase in HMGB1 levels in the cells results in sensitization of the cells to cisplatin (19). This sensitization effect is most likely a consequence of the interaction between HMGB1 and cisplatin-damaged DNA, which may interfere with the repair of platinum-DNA adducts and exacerbate the inhibitory effects of cisplatin damage on DNA replication and transcription. The clonogenic assays presented here reveal that BG-1 cells are sensitized to cis-[Pt(NH3)(NH2Cy)Cl] following steroid hormone treatment in a manner similar to their sensitization to cisplatin, whereas hormone treatment had no effect on the sensitivity of the cells to [Pt(dach)Cl2] (Fig. 5). These results suggest that, in BG-1 cells, HMGB1 interacts with cis-[Pt(NH3)(NH2Cy)]^2+-damaged DNA and influences the cellular processing of cis-[Pt(NH3)(NH2Cy)]^2+-DNA adducts in a manner similar to its effect on cisplatin-damaged DNA. On the other hand, there is most likely no strong interaction between HMG box proteins and [Pt(dach)]^2+-DNA lesions; thus, overexpression of HMGB1 by hormone treatment does not sensititize the cells to [Pt(dach)Cl2]. The cell sensitization results are consistent with those of the binding of HMGB1a to dsTG*G*A probes as revealed by EMSA but inconsistent with the binding of HMGB1a and HMGB1 (23) to dsAG*G*C oligonucleotides. These results further highlight the importance of flanking sequence in studies for understanding the recognition of platinum-DNA lesions by cellular proteins. Although the differences between binding affinity of HMGB1a to various platinum-DNA adducts presented here cannot be directly extrapolated to that of HMGB1, the good agreement of our data on HMGB1a binding to the previous result on HMGB1 binding indicates that HMGB1a mediate the different interactions between HMGB1 and platinum-damaged DNA.
DNA. The consistency between the cellular sensitization and the binding affinity of HMGB1a for cisplatin-, (Pt(dach))²⁺- and cis-(Pt(NH₃)₂(NH₂Cy))²⁺-damaged DNA also supports this assumption.

Concluding Remarks—This work reveals the consequences of variation in spectator ligands on the binding of HMG box proteins and TBP to DNA containing 1,2-intrastand platinum d(GpG) cross-links. Electrophoretic mobility shift assays show that both domains of HMGB1 and TBP discriminate between platinum-DNA adducts containing different ligands in a similar fashion, with HMGB1a exhibiting the highest selectivity. The effect of spectator ligands on protein binding is also highly dependent on the base pairs flanking the modified d(GpG) site. Thus, studies that investigate protein binding to platinum-modified DNA, particularly with regard to potentially novel platinum anti-tumor agents, should include the effects of sequence context. Our results highlight the need to develop methods for examining the importance of sequence context on protein-DNA interactions in cells.

Acknowledgments—We thank Dr. E. R. Jamieson for providing the HMGB1 domains and Johnson Matthey for the gift of cisplatin.

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