The TATA-binding protein (TBP) recognizes the TATA box element of transcriptional promoters and recruits other initiation factors. This essential protein binds selectively to cisplatin-damaged DNA. Electrophoretic mobility shift assays were performed to study the kinetics of TBP binding both to the TATA box and to cisplatin-damaged DNA in different sequence contexts. TBP binds with high affinity ($K_d = 0.3$ nM) to DNA containing sitespecific cisplatin 1,2-intrastrand (dGpG) cross-links. The $k_{on}$ and $k_{off}$ values for the formation of these TBP complexes are $1-3 \times 10^6$ M$^{-1}$ s$^{-1}$ and $\sim 1-5 \times 10^{-4}$ s$^{-1}$, respectively, similar to the corresponding values for the formation of a TBP-TATA box complex. In electrophoretic mobility shift assay competition assays, cisplatin-damaged DNA extensively sequesters TBP from its natural binding site, the TATA box. Nine DNA probes were prepared to determine the flanking sequence dependence of TBP binding to cisplatin-modified DNA. TBP clearly displays sequence context selectivity for platinated DNA, very similar to but not as dramatic as that of the high mobility group protein HMGB1. When TBP was added to an in vitro nucleotide excision repair assay, it specifically shielded cisplatin-modified 1,2-(GpG) intrastrand cross-links from repair. These results indicate that TBP is likely to be a key protein in mediating the cytotoxicity of cisplatin.

After the discovery of the anticancer activity of cisplatin, many studies have focused on elucidating its mechanism of action (1, 2). The formation of covalent cisplatin-DNA adducts, especially the 1,2-intrastrand (dGpG) cross-link, correlates with the cytotoxicity of the drug (1–3). Attention now focuses on understanding how cells react to the presence of the cisplatin-DNA lesions and, using this information, designing more effective anticancer treatments. Upon cisplatin binding, the DNA duplex is bent and unwound, and the minor groove becomes wide and shallow. These structural changes inhibit essential DNA metabolic processes such as replication and transcription (4–7). The distorted DNA duplex can also interact with a number of cellular proteins (1, 2, 8, 9), an activity postulated to mediate the processing of cisplatin-DNA lesions (1–3, 10–14).

The identification and characterization of proteins that bind selectively to cisplatin-damaged DNA has therefore become one of the main thrusts of research in this field.

The TATA-binding protein (TBP) is a key component of transcription factor IID, which is required for transcription initiation of all three eukaryotic RNA polymerases (15). As the first step in the process, TBP recognizes a TATA box element located $\sim 30$ base pairs (bp) upstream from the transcription start site and eventually recruits other transcription factors. Structural analyses of several TBP-TATA box complexes reveal that TBP binds at a widened minor groove and bends the duplex DNA toward the major groove by intercalation of two pairs of phenylalanine residues (16–18). Because sequence-specific DNA-binding proteins usually reside in the major groove, the minor groove binding by TBP was initially puzzling. Subsequent studies demonstrated that the flexibility of the TATA box element primarily determines its binding affinity to TBP (19, 20). In addition to the specificity of its DNA binding, TBP has distinctive DNA binding kinetics. The formation of the TBP-DNA complex is characterized by relatively slow on and off rates (21).

TBP binds selectively to cisplatin-damaged DNA (22). The sequestration of TBP by cisplatin DNA adducts inhibits transcription in vitro, which could be restored in a reconstituted system by addition of extra TBP (22). Transcription inhibition by exogenously added cisplatin-damaged DNA has also been reported (23). More recently, enhanced binding of TBP to the TATA element containing flanking cisplatin 1,2-intrastrand cross-links was demonstrated (24). This series of experiments suggests a possible role for TBP-cisplatin-DNA ternary complexes in mediating the anticancer activity of the drug.

Because a variety of proteins interact with cisplatin-DNA adducts (9), many studies have focused on determining their binding affinities (25–30). To evaluate the relative importance of the different protein-platinated DNA interactions in the cell, however, detailed kinetic parameters are also required. Despite the importance of such information, few attempts have been made to examine the kinetics of protein binding to cisplatin-damaged DNA (31–33). The interactions between the two domains (A and B) of HMGB1 with cisplatin-modified DNA were investigated by using stopped-flow fluorescence and fluorescence resonance energy transfer methods (31, 32). In addition, a stopped-flow kinetic analysis was performed for replication protein A (RPA) binding to cisplatin-damaged DNA (33).

In the present study we employed electrophoretic mobility

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shift assays (EMSA) were used to examine the kinetics of TBP binding to cisplatin-damaged DNA and, for comparison purposes, the TATA box. TBP binding to DNA containing a site-specific 1,2-intrastrand d(GpG) cross-link was evaluated by using several platinum-modified DNA probes with various flanking sequences. We also performed an in vitro repair assay to examine the effect of TBP in modulating nucleotide excision repair (NER) of platinated DNA. The results provide strong evidence for a biological role of TBP in mediating the cytotoxicity of cisplatin.

**EXPERIMENTAL PROCEDURES**

Preparation of Oligonucleotides Probes—Table 1 lists the 25-bp oligonucleotides used in this study together with their abbreviations. The synthesis, platination, and purification of site-specifically platinated single-stranded oligonucleotides were carried out as described previously (26). Platinated top strands, (5′-CCCTCCTCTCCNGGNGN2TCTT-CTCTCC-3′, N2 = A, T, or C), where the asterisks indicate the formation of Pt(N7) bonds, were annealed with their complementary bottom strands in 10 mM Tris (pH 7.0), 50 mM NaCl, and 10 mM MgCl2, heated to 90 °C, and slowly cooled to 4 °C over several hours. The resulting cisplatin-modified duplex probes were purified by ion-exchange HPLC using a Dionex DNApac PA-100 column. These oligonucleotides were desalted by dialysis and concentrated to 5–10 μM. The TATAMLP probe (Table 1) was prepared by the same method except for the platination step. The TATA element of adenovirus major late promoter, one of the strongest promoters, was used for this study. The purity of the platinated single-stranded DNA was confirmed by analytical HPLC. Atomic absorption spectroscopy combined with UV-visible spectroscopy and electrospray mass spectrometry verified the existence of singly platinated probes (see the supplemental material on-line).

EMSA—All platinated and TATAMLP duplex probes (~10 pmol) were radioactively labeled at their 5′-ends by using 50 μCi of [γ-32P]ATP (PerkinElmer Life Sciences) and 20 units of polyol nucleotide kinase (New England Biolabs). Labeled probes were separated from small nucleotides by passage through a G-25 Sephadex Quickspin columns (Roche Molecular Biochemicals). For each binding reaction, DNA probes (0.5–2 nM, ~10,000 cpm) and the indicated concentrations of TBP were mixed and incubated in a buffer solution containing 60 mM KCl, 20 mM Tris (pH 7.9), 5 mM MgCl2, 10 mM dithiothreitol, 0.2 mg/ml bovine serum albumin, and 10% glycerol (24). After incubation at 30 °C for 30 min, binding mixtures were directly loaded onto 6% native polyacrylamide gels and electrophoresed for 1.5 h at 150 V in 25 mM Tris (pH 7.9), 190 mM glycine, 1.0 mM EDTA, and 40 mM MgCl2 running buffer. The gels were dried at 80 °C and then exposed to phosphorimaging plates for 15–20 h. The bands were quantified using ImageMaster 2D software (GE Healthcare Life Sciences). The bands were analyzed by phosphorimaging and by staining the gels with ethidium bromide and scanning with a gel scanner (Bio-Rad).

Kinetic EMSA Analysis—For the measurement of the protein-DNA association constants by kinetic methods, the increase in the amount of complex was monitored at various times after the addition of 5–10 nM TBP to ~1 nM probe. As described previously (35), the raw data were fit to Equation 1,

$$I/[TBP]/ln([probe]/([probe] - [complex])] = k_{on}t$$  \hspace{1cm} (Eq. 1)

where $I$ is the intensity of the band, $[TBP]$ is the concentration of TBP, [probe] is the concentration of probe, and [complex] represents the complex concentration at time $t$. The left side of the equation was plotted against time in $s$, and the $k_{on}$ value for each probe was obtained from the slope of the plot. In the equation, [TBP], [probe], and [complex] indicate the initial concentrations of TBP and DNA probe, respectively, and [complex] is the concentration of complex at each time point.

To determine the dissociation rate constants ($k_{off}$), ~10 nM TBP and ~1 nM probe were mixed at 30 °C for 30 min to reach the equilibrium state. Dissociation of the protein-DNA complexes was initiated by the addition of 200 ng of poly[d(IdC)] at different time points to assure that all dissociation reactions were complete at the same time. The decrease in the amount of complex was followed over a 1- or 2-h time period. The data were fit to Equation 2 to obtain the first-order rate constant for the dissociation reaction,

$$ln([complex]/[complex]_0) = -k_{off}t$$  \hspace{1cm} (Eq. 2)

where [complex] indicates the concentration of complex at time $t$, and [complex]$_0$ is the complex concentration under the initial binding conditions. The natural logarithm of [complex] divided by [complex]$_0$ was plotted versus time, and the negative slope of the fit provided the $k_{off}$ value. The dissociation constant, $K_d$, values were calculated from these results ($K_d = k_{off}/k_{on}$).

Competition Assay—Competitor DNA of varying concentrations was mixed with a fixed amount of TBP and radioactively labeled TATAMLP, and the disappearance of the TBP-TATAMLP complex was analyzed by EMSA. Competition assays were also used to obtain the $K_d$ values for weak binding probes. In such a competition experiment, two binding reactions proceed simultaneously and follow the relationship in Equation 3 (37),

$$\theta = \frac{P_1(1 - \theta)}{K_r(1 + C_r/K_r) + T_1(1 - \theta)}$$  \hspace{1cm} (Eq. 3)

where $P_r$, $C_r$, and $T_r$ represent the total concentrations of TBP, competitor, and TATAMLP, respectively. $K_r$ and $K_c$ are the respective dissociation constants of TATAMLP and competitor. At different $C_r$ values, the binding fraction ($\theta$) was determined. The $K_d$ value can be calculated by using Equation 4, where $C_{1/2}$ represents the concentration of competitor at $\theta = 1/2$ (37).

$$K_d = \frac{2KC_{1/2}}{P_1 - T_1 - 2KC_{1/2}}$$  \hspace{1cm} (Eq. 4)

Excision Repair Assay—Whole cell-free extracts (CFE) from HeLa cells were prepared by a reported method (38) and stored at ~80 °C. DNA duplexes 161 bp in length containing a site-specific cisplatin 1,2-d(GpG) or 1,3-d(GpTpG) cross-link with a radiolabeled phosphate located six or seven bases to the 5′ side of the cisplatin binding site were prepared as previously described (39, 40). The probes contained three phosphorothioate residues at their 3′ ends to minimize nonspecific nuclelease degradation. Whole cell extracts were incubated with damaged DNA in excision repair buffer (39), and excision fragments were resolved by 10% denaturing polyacrylamide gel electrophoresis. For the repair experiment in the presence of TBP, the protein was pre-incubated with the damaged DNA probe in excision repair buffer for 30 min on ice unless otherwise indicated. The extent of NER was measured by comparing the signal intensity corresponding to the 25–30-bp excised fragment with that of the entire lane. The small amount of DNA degradation due to nonspecific nuclease activity was subtracted from the repair signal by using the area corresponding to the 32–37-bp region as background (41).

**RESULTS**

Kinetics of TBP Binding to the TATA Box and Cisplatin-damaged DNA—To optimize conditions for the EMSA experiments, probes of three different lengths (15, 25, and 35 bp) were investigated. The 15-bp probe showed weak binding affinity to TBP compared with longer probes. In addition, this short oligonucleotide was partially melted under the experimental conditions and did not exhibit any nonspecific binding. Therefore, 25-bp probes were used for further studies.

EMSA analyses were performed to investigate the kinetics of TBP binding to the TATA box (TATAMLP) and cisplatin-damaged DNA (AGGA, Table 1). Fig. 1A shows EMSA data used to determine the association rate constants for two probes, and the data analysis is presented in Fig. 1B. The bimolecular binding reactions reach the equilibrium state within 10 min for both probes. As shown in Fig. 1A, however, the TBP-AGGA complex clearly forms more rapidly than the TBP-TATAMLP complex. Data collected within 2–3 min were fit to Equation 1, yielding $k_{on}$ values of $1.3 \times 10^5$ M$^{-1}$ s$^{-1}$ for TBP binding to TATAMLP and $3.0 \times 10^3$ M$^{-1}$ s$^{-1}$ for the AGGA probe (Fig. 1B). The $k_{on}$ value for TATAMLP is in good agreement with published values, which fall in the range 1.0–3.0 x 10$^5$ M$^{-1}$ s$^{-1}$ (21, 35, 42–44).

After the addition of excess of poly[d(IdC)] to the TBP-DNA...
complex, decreased amounts of the complex were detected (Fig. 2). The dissociation reaction data were fit to Equation 2. The AGGA and TATAML probe dissociate from TBP with half-lives, $t_{1/2}$, of 120 min and 190 min, respectively. Although TBP associates with AGGA more rapidly than with TATAML, the latter probe has a slightly lower off-rate (Table II).

From these $k_{on}$ and $k_{off}$ results, dissociation constants $K_d (k_{off}/k_{on})$ for each probe were calculated (Table II). Comparison of $K_d$ values for AGGA and TATAML reveals that cisplatin-damaged DNA has about a 1.5-fold higher binding affinity to TBP compared with that of its natural binding site, the TATA box, under the same binding conditions. The $K_d$ value (0.44 nM) for the TATA box binding to adenovirus major late promoter is consistent with previous reports (21, 34, 35, 42–45).

Flanking Sequence Preference of TBP Binding to Cisplatin-DNA—Previously we demonstrated that HMG domains display a distinct selectivity for platinated DNA containing different flanking sequences (26, 46). Moreover, different flanking sequences can affect the conformational and thermodynamic properties of cisplatin-damaged oligonucleotides (47). Here, we studied the kinetics of TBP binding to cisplatin-damaged probes in diverse sequence contexts (Table I). Fig. 3

![Figure 1](image1.png)

**FIG. 1.** EMSA experiment to determine $k_{on}$. A, TBP-AGGA and TBP-TATAML probe dissociations are represented in the top and bottom autoradiographs, respectively. Free DNA and the TBP complex are shown at various time points and are indicated by arrows. B, the binding fraction ($\theta$) for AGGA (closed circles) and TATAML (open circles) are plotted versus time. Lines were obtained by fitting the raw data within 200 s to Equation 1 shown in the inset.

![Figure 2](image2.png)

**FIG. 2.** EMSA experiment to determine $k_{off}$. A, TBP-AGGA and TBP-TATAML dissociation reactions are represented in the top and bottom autoradiographs, respectively. $B$, the natural logarithm of the complex concentration divided by the initial concentration at $t = 0$ is plotted versus time. Lines were plotted according to Equation 2.

| Probe         | $k_{on}$ | $k_{off}$ | $K_d$ |
|---------------|----------|-----------|-------|
| TATAML        | $2.1 \pm 0.2 \times 10^5$ | $1.9 \pm 0.2 \times 10^5$ | 0.44  |
| AGGA          | $2.0 \pm 0.1 \times 10^5$ | $2.0 \pm 0.1 \times 10^5$ | 0.30  |
| AGGT          | $1.3 \pm 0.1 \times 10^5$ | $1.3 \pm 0.1 \times 10^5$ | 0.48  |
| TGGT          | $2.0 \pm 0.1 \times 10^5$ | $2.0 \pm 0.1 \times 10^5$ | 2.8   |
| TGGC          | $2.0 \pm 0.1 \times 10^5$ | $2.0 \pm 0.1 \times 10^5$ | 2.8   |
| CGGA          | $2.0 \pm 0.1 \times 10^5$ | $2.0 \pm 0.1 \times 10^5$ | 1.9   |
| CGGT          | $2.0 \pm 0.1 \times 10^5$ | $2.0 \pm 0.1 \times 10^5$ | 1.9   |
| CGGC          | $2.0 \pm 0.1 \times 10^5$ | $2.0 \pm 0.1 \times 10^5$ | 1.9   |

$^a$ Measured by competition assay (see “Experimental Procedures”).
shows EMSAs of TBP binding to TATAMLP and nine different cisplatin-damaged probes. Sequence selectivity is clearly manifest for TBP binding to platinated DNA.

Kinetic EMSA experiments were performed to determine $k_{on}$ and $k_{off}$ values for each probe. Fig. 4A indicates fits to EMSA data obtained from the association kinetics for six different cisplatin-damaged probes. All $k_{on}$ values lie between $2.1 \times 10^5 \text{ M}^{-1} \text{s}^{-1}$ and $1.2 \times 10^5 \text{ M}^{-1} \text{s}^{-1}$, indicating that most of the cisplatin-damaged DNA complexes have faster association rates than that of the TATA box (Table II). The $k_{off}$ values were also calculated by fitting the dissociation EMSA data to Equation 2 (Fig. 4B). The two more weakly binding probes, TGGC and CGGC, did not allow reliable kinetic data to be obtained. We therefore carried out competition assays to obtain relative $K_d$ values for these two probes. At various concentrations of TGGC or CGGC competitor, the binding fraction of the TBP to TATAMLP was examined by EMSA and quantitated (Fig. 5).

Our results reveal a clear flanking sequence dependence of TBP binding to cisplatin-damaged DNA (Table II). At the N1 position, TBP prefers dA rather than T or dC, and TBP forms more stable complexes when N2 is either dA or T.

Cisplatin-damaged DNA Sequesters TBP from the TATA Box—A DNase I footprinting assay previously demonstrated reduced binding of TBP to the TATA box after the addition of excess cisplatin-damaged DNA (48). In the present study, competition EMSA experiments were performed to study the relative binding affinities of TBP to the TATA box and cisplatin-damaged DNA. TBP and the two DNA probes were mixed, and the amounts of free and bound TATA box were analyzed by using radioactively labeled TATA box DNA. Fig. 6 provides clear evidence that TBP dissociates from the TATA box upon addition of cisplatin-damaged DNA. At 1 nM TBP, more than half of the TBP-TATAMLP complex has dissociated when equivalent amounts (4 nM) of AGGA and TATAMLP are present. Upon the addition of a 3-fold excess of AGGA, most of the TBP is sequestered from the natural TATAMLP binding site.

TBP Blocks NER of the Cisplatin 1,2-d(GpG) Cross-link—Cisplatin-modified DNA is repaired by the NER machinery. When HMG-domain proteins are added to HeLa cell-free extracts or reconstituted NER components, a significant amount of the repair of cisplatin 1,2-d(GpG) intrastrand cross-links is inhibited (39). Repair of cisplatin 1,3-d(GpTpG) intrastrand cross-links, which do not bind HMG-domain proteins, was not significantly affected under the same conditions. Because TBP has a high binding affinity for cisplatin 1,2-d(GpG) intrastrand-cross-linked DNA, we next examined repair of this ad-
duct by NER using HeLa whole cell-free extracts in the presence of TBP.

For this experiment we employed the CGGC probe because its repair signal is larger by a factor of two than that of AGGA (data not shown). Fig. 7 reveals that repair of the cisplatin 1,2-d(GpG) intrastrand cross-link in CGGC is indeed inhibited by preincubation with TBP, whereas a probe containing the cisplatin 1,3-d(GpTpG) intrastrand cross-link is not. In the presence of a great excess of TBP (>200 μM), repair of the cisplatin 1,3-d(GpTpG) cross-link is also shielded to a similar extent as the 1,2-d(GpG) cross-link, probably because of nonspecific binding (data not shown).

**DISCUSSION**

**TBP Binding to Cisplatin-damaged DNA**—The present study reveals that TBP very slowly associates with and dissociates from cisplatin 1,2-d(GpG) intrastrand cross-links, behavior that is very similar to its binding to the TATA box. Including these results, kinetic parameters for the binding of three proteins, HMGB1-domain proteins, TBP, and RPA, to cisplatin-modified DNA are now available (31–33). Although it is hard to compare the kinetic parameters directly, because of the different binding conditions and temperatures employed, TBP clearly exhibits very different properties for the binding to cisplatin-damaged DNA. The $k_{on}$ values reveal that the HMGB1 domains bind to cisplatin-modified DNA near the diffusion limit ($k_{on} = 5 \times 10^8 \text{M}^{-1} \text{s}^{-1}$) more than 1000-fold more rapidly than the association of TBP ($k_{on} = 3.0 \times 10^9 \text{M}^{-1} \text{s}^{-1}$). RPA also has about a 100-fold higher association rate constant ($k_{on} = 2.2 \times 10^6 \text{M}^{-1} \text{s}^{-1}$) than TBP. If the association rate were the determining factor in protein binding to target lesions in the cell, TBP would be an unlikely participant in the competition for cisplatin-modified DNA. However, HMGB1 domains and RPA also dissociate much more rapidly than the extremely stable TBP-platinated DNA complex. Under relatively similar binding conditions, TBP has more than a 100-fold higher binding affinity for cisplatin-damaged DNA than RPA (33). In addition, TBP has the highest specificity for cisplatin-damaged DNA among all the identified proteins (1). Because the $K_d$ for TBP binding to genomic DNA is $6 \times 10^{-6}$ M, this protein prefers to bind platinum-damaged DNA by a factor of $>3000$ compared with undamaged DNA (34). RPA and HMGB1 have $-15$- and 100-fold preferences for damaged over undamaged DNA, respectively (25, 28, 49).

Several protein-protein interactions involving TBP, RPA, and HMGB1 occur that can affect the DNA binding kinetics in the cell. XPA, another damage recognition protein, stabilizes the RPA-damaged DNA complex (28, 50). Interactions between human TBP and HMGB1 occur that can affect the DNA binding kinetics in the cell, TBP would be an unlikely participant in the competition for cisplatin-modified DNA. However, HMGB1 domains and RPA also dissociate much more rapidly than the extremely stable TBP-platinated DNA complex. Under relatively similar binding conditions, TBP has more than a 100-fold higher binding affinity for cisplatin-damaged DNA than RPA (33). In addition, TBP has the highest specificity for cisplatin-damaged DNA among all the identified proteins (1). Because the $K_d$ for TBP binding to genomic DNA is $6 \times 10^{-6}$ M, this protein prefers to bind platinum-damaged DNA by a factor of $>3000$ compared with undamaged DNA (34). RPA and HMGB1 have $-15$- and 100-fold preferences for damaged over undamaged DNA, respectively (25, 28, 49).

**Flanking Sequence Dependence of TBP Binding to Cisplatin-damaged DNA**—Kinetic analyses of TBP binding to 10 different probes were performed, and the results are summarized in Table II. The trends in the kinetically determined $K_d$ values of the individual probes agree well with the EMSA data in Fig. 3. Both $k_{on}$ and $k_{off}$ values generally affect the binding affinities of the probes studied. This binding is unlike that encountered in classical TATA box kinetic studies in which different TATA boxes have different $k_{on}$ values (24, 35, 44). Specifically, it is the differences in $k_{off}$ values that mainly determine the relative TBP binding affinities between the three probes AGGA, AGGT, and AGGC.

The flanking sequence dependence of TBP binding to cispla-
Kinetic Studies of TBP Interaction with Cisplatin-DNA

Fig. 7. Effect of TBP on excision repair assay of cisplatin-DNA intrastrand cross-links in HeLa cell extract. The substrates were preincubated for 30 min before the addition of HeLa CFEs. A, 10% denaturing gel demonstrating the diminishing repair signal by TBP. B, densitometric quantification of the data. Relative repair levels are plotted against the concentration of TBP added using 1,2-d(GpG) (circles) and 1,3-d(GpTpG) (triangles) intrastrand cross-links as repair probes. Error bars represent 1 S.D. The absolute excision levels in the absence of TBP were 0.2 ± 0.05% for the 1,2-d(GpG) intrastrand cross-link and 2.0 ± 0.5% for the 1,3-d(GpTpG) intrastrand cross-link. nt, nucleotides.

Fig. 8. Schematic diagram of TBP-DNA complex interactions as revealed by footprinting. A, TBP-TATAMLP interaction. Boldface indicates the conserved TATA box. Intercalating phenylalanine residues and hydrogen-bonding contact regions (19) are indicated by arrows and closed circles, respectively. B, TBP-cisplatin-damaged DNA interaction. Boldface indicates the cisplatin d(GpG) lesion and flanking sequences. Dotted and solid braces show regions protected by TBP from DNase I (48) and hydroxyl radical (this study), respectively.

Despite the similarity in flanking sequence preferences, TBP is much less selective than HMGB1 domain A. The highest affinity probe, AGGA, has only 40-fold lower $K_d$ values compared with the weakest (TGGC) probe for the interaction with TBP. In contrast, the binding affinities of HMGB1 domain A vary by several orders of magnitude depending upon the sequence context of the cisplatin-DNA lesion, and the lowest affinity probe has only severalfold higher binding affinity than that of undamaged DNA (26). Because different flanking sequences have the same likelihood of occurring in platinated DNA (57), some cisplatin DNA lesions may not participate at all in interactions with HMGB1 in the cell. A crystal structure of HMGB1 domain A bound to a cisplatin-modified DNA duplex revealed the intercalation of a single phenylalanine residue into the hydrophobic wedge created in the minor groove across
from the platinum-induced d(GpG) cross-link, with its binding extending mainly to 3’ side of the adduct (58). Because this intercalation interaction is critical for tight binding of HMG1-domain A, the flanking sequence significantly affects the binding affinity of the protein to cisplatin-modified DNA (26). In contrast, in the TBP complex, the cisplatin d(GpG) adduct is located between the two intercalation sites, resulting in a much less significant flanking sequence influence on binding affinity. Although the two proteins have different degrees of selectivity for flanking sequence, their preference trends are similar. In both cases, flexibility at the flanking base pairs is a primary contributor to binding selectivity.

**Biological Implications of TBP Binding to Cisplatin-damaged DNA**—In the cell the TBP-TATA interaction is required for the recognition and utilization of eukaryotic promoters in basal transcription. The formation rate and stability of TBP-TATA complexes are major determinants that regulate transcription (43, 59, 60). Disruption of this important interaction is an attractive candidate for explaining the cytotoxicity of cisplatin.

In the present investigation, one of the site-specifically cisplatin-modified probes, AGGAg, showed a $K_d$ value of 0.3 nM, which indicates 1.5-fold higher binding affinity to TBP than a strong TATA element. Such high binding affinity of cisplatin-modified DNA for TBP suggests that the drug can interfere with cellular TBP-TATA interactions. Furthermore, cisplatin lesions with different flanking sequences show similarly high binding affinity to TBP (Table II), as discussed above. Even the most weakly binding probe, TGGC, has sufficient binding affinity ($K_d = 12$ nM) to disturb the TBP-TATA interaction, because many weak promoters have TATA elements with $K_d$ values higher than 12 nM (19, 43). In the mammalian genome, ~10,000 transcribed genes contain the TATA box in their promoters (2, 62, 63). These data and our kinetic results suggest that the hijacking of TBP from its natural binding site, the TATA element, might contribute to the cytotoxicity of cisplatin.

NER is a major pathway for removing DNA lesions including those of cisplatin (64). Specific interactions between cisplatin-modified DNA and damage-detection components of NER such as XPA and RPA have been extensively investigated (65–67). In addition, a study demonstrated that diminished repair in tumor cells sensitizes them to cisplatin (68). Apart from the NER components, there has been much interest in the role of other cellular proteins binding to cisplatin-modified DNA. Previous work revealed that HMGB1 proteins block cisplatin lesions from NER, as monitored by a repair assay conducted with previous work revealed that HMGB1 proteins block cisplatin lesions from NER, as monitored by a repair assay conducted with

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