Different Recognition of DNA Modified by Antitumor Cisplatin and Its Clinically Ineffective \textit{trans} Isomer by Tumor Suppressor Protein p53\textsuperscript{*}

Jana Kasparková\textsuperscript{‡§}, Sarka Pospisilova\textsuperscript{†}, and Viktor Brabec\textsuperscript{¶¶}

From the \textsuperscript{‡}Institute of Biophysics, Academy of Sciences of the Czech Republic, CZ-61265 Brno, Czech Republic and the \textsuperscript{¶}Masaryk Memorial Cancer Institute, CZ-66533 Brno, Czech Republic

The p53 gene encodes a nuclear phosphoprotein that is biologically activated in response to genotoxic stresses including treatment with anticancer platinum drugs. The DNA binding activity of p53 protein is crucial for its tumor suppressor function. DNA interactions of active wild-type human p53 protein with DNA fragments and oligodeoxyribonucleotide duplexes modified by antitumor cisplatin and its clinically ineffective \textit{trans} isomer (transplatin) were investigated by using a gel mobility shift assay. It was found that DNA adducts of cisplatin reduced binding affinity of the consensus DNA sequence to p53, whereas transplatin adducts did not. This result was interpreted to mean that the precise steric fit required for the formation and stability of the tetrameric complex of p53 with the consensus sequence cannot be attained, as a consequence of severe conformational perturbations induced in DNA by cisplatin adducts. The results also demonstrate an increase of the binding affinity of p53 to DNA lacking the consensus sequence and modified by cisplatin but not by transplatin. In addition, only major 1,2-GG intrastrand cross-links of cisplatin are responsible for this enhanced binding affinity of p53. The data base on structures of various DNA adducts of cisplatin and transplatin reveals distinctive structural features of 1,2-intrastrand cross-links of cisplatin, suggesting a unique role for this adduct in the binding of p53 to DNA lacking the consensus sequence. The results support the hypothesis that the mechanism of antitumor activity of cisplatin may also be associated with its efficiency to affect the binding affinity of platinated DNA to active p53 protein.

It is well established that platinum coordination complexes exhibit antitumor effects (1, 2). The success of platinum complexes in killing tumor cells results from their ability to form on DNA various types of covalent adducts (3). The first platinum complex introduced in the clinic is \textit{cis}-diaminedichloroplatini-1

\textsuperscript{*} This work was supported in part by the Grant Agency of the Czech Republic (Grants 205/99/0695, 205/01/0418, and 301/00/P094), the Grant Agency of the Academy of Sciences of the Czech Republic (Grant A5004101), and the Internal Grant Agency of the Ministry of Health of the Czech Republic (Grant NL6058–3/2000). 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.

\textsuperscript{§} Supported in part by an International Research Scholar’s award from the Howard Hughes Medical Institute and the Wellcome Trust (United Kingdom).

\textsuperscript{¶} To whom correspondence should be addressed: Institute of Biophysics, Academy of Sciences of the Czech Republic, Královopolska 135, CZ-61265 Brno, Czech Republic. Tel.: 420-5-41517148; Fax: 420-5-41240499; E-mail: brabec@ibp.cz.

\textsuperscript{1} The abbreviations used are: cisplatin, \textit{cis}-diaminedichloroplatinum(II); CL, cross-link; transplatin, \textit{trans}-diaminedichloroplatinum(II); wt, wild-type; bp, base pair(s); CDRE, consensus DNA response element; \{Cl(dien)Pt\}Cl, diethylenetriaminechloroplatinum(II) chloride; PAGE, polyacrylamide gel electrophoresis; mAb, monoclonal antibody; HMG, high mobility group.
related to its DNA binding activity. Active wt p53 binds as a tetramer to over 100 different response elements naturally occurring in the human genome. These response elements, which show functionality, differ in the details of their specific base sequence, but all contain two tandem consensus decamers, each a pentameric inverted repeat. Most consensus decamers, separated in the binding unit by 0–21 base pairs (bp), follow the consensus sequence pattern (17) PuPuPuPu(C/A)(T/A)GPpPyPy, where Pu and Py are purines and pyrimidines, respectively, and the vertical bar denotes the center of pseudodyad symmetry. Four molecules of the DNA-binding domain of p53 bind the response elements with high cooperativity. They also bend DNA. It has been suggested (18) that this bending is localized mostly at the two pentamer CA/TG junctions in the consensus DNA response element (CDRE) (by 25–28° at each junction) toward the major groove. Active wt p53 also over-twists the DNA response element by ~70°. This DNA twisting is uniformly distributed among the pentamers. It has also been suggested that due to many functions of p53 protein the demands for binding specificity and selectivity are necessarily extraordinary, which is accomplished through its tetrameric association with a repetitive binding site. Precise steric fit accommodated through both DNA bending and twisting appears extremely important in this binding site. Because DNA bending and twisting are coupled in the p53-DNA complex, the bending specificity of the p53 system as well as complex stability could be fine-tuned by agents that affect DNA bending and twisting.

It has been shown (3) that DNA adducts of cisplatin and other platinum bifunctional compounds distort the conformation of DNA, including bending and changes in the twist angle. Thus, formation of the adducts by platinum compounds in the CDRE could affect its binding affinity to p53. No direct interaction between active wt p53 and platinum-modified DNA in cell-free media has yet been reported. Only binding of latent p53 protein, lacking sequence-specific DNA binding, to DNA modified by cisplatin has been reported (19). In the present work, we have used gel mobility shift assay methodology to investigate the binding affinity of the active human wt p53 system to the CDRE modified by either antitumor cisplatin or its clinically inefficient trans isomer in a cell-free medium. We have also examined binding of DNA lacking CDRE and modified by cisplatin or transplatin to reveal affinity of active wt p53 to platinated DNA containing no consensus nucleotide sequence. Thus, these studies could provide insight into the relative cytotoxicities of these two isomers, thereby potentially aiding in the rational design of new platinum drugs as well as illuminating aspects of the role of p53 in chemotherapy by its DNA binding activity. Active wt p53 binds to platinated DNA containing no consensus nucleotide sequence, but all contain two tandem consensus decamers, each a pentameric inverted repeat. Most consensus decamers, separated in the binding unit by 0–21 base pairs (bp), follow the consensus sequence pattern (17) PuPuPuPu(C/A)(T/A)GPpPyPy, where Pu and Py are purines and pyrimidines, respectively, and the vertical bar denotes the center of pseudodyad symmetry. Four molecules of the DNA-binding domain of p53 bind the response elements with high cooperativity. Four molecules of the DNA-binding domain of p53 bind the response elements with high cooperativity. They also bend DNA. It has been suggested (18) that this bending is localized mostly at the two pentamer CA/TG junctions in the consensus DNA response element (CDRE) (by 25–28° at each junction) toward the major groove. Active wt p53 also over-twists the DNA response element by ~70°. This DNA twisting is uniformly distributed among the pentamers. It has also been suggested that due to many functions of p53 protein the demands for binding specificity and selectivity are necessarily extraordinary, which is accomplished through its tetrameric association with a repetitive binding site. Precise steric fit accommodated through both DNA bending and twisting appears extremely important in this binding site. Because DNA bending and twisting are coupled in the p53-DNA complex, the bending specificity of the p53 system as well as complex stability could be fine-tuned by agents that affect DNA bending and twisting.

It has been shown (3) that DNA adducts of cisplatin and other platinum bifunctional compounds distort the conformation of DNA, including bending and changes in the twist angle. Thus, formation of the adducts by platinum compounds in the CDRE could affect its binding affinity to p53. No direct interaction between active wt p53 and platinum-modified DNA in cell-free media has yet been reported. Only binding of latent p53 protein, lacking sequence-specific DNA binding, to DNA modified by cisplatin has been reported (19). In the present work, we have used gel mobility shift assay methodology to investigate the binding affinity of the active human wt p53 system to the CDRE modified by either antitumor cisplatin or its clinically inefficient trans isomer in a cell-free medium. We have also examined binding of DNA lacking CDRE and modified by cisplatin or transplatin to reveal affinity of active wt p53 to platinated DNA containing no consensus nucleotide sequence. Thus, these studies could provide insight into the relative cytotoxicities of these two isomers, thereby potentially aiding in the rational design of new platinum drugs as well as illuminating aspects of the role of p53 in chemotherapy by its DNA binding activity. Active wt p53 binds to platinated DNA containing no consensus nucleotide sequence, but all contain two tandem consensus decamers, each a pentameric inverted repeat. Most consensus decamers, separated in the binding unit by 0–21 base pairs (bp), follow the consensus sequence pattern (17) PuPuPuPu(C/A)(T/A)GPpPyPy, where Pu and Py are purines and pyrimidines, respectively, and the vertical bar denotes the center of pseudodyad symmetry. Four molecules of the DNA-binding domain of p53 bind the response elements with high cooperativity. Four molecules of the DNA-binding domain of p53 bind the response elements with high cooperativity. They also bend DNA. It has been suggested (18) that this bending is localized mostly at the two pentamer CA/TG junctions in the consensus DNA response element (CDRE) (by 25–28° at each junction) toward the major groove. Active wt p53 also over-twists the DNA response element by ~70°. This DNA twisting is uniformly distributed among the pentamers. It has also been suggested that due to many functions of p53 protein the demands for binding specificity and selectivity are necessarily extraordinary, which is accomplished through its tetrameric association with a repetitive binding site. Precise steric fit accommodated through both DNA bending and twisting appears extremely important in this binding site. Because DNA bending and twisting are coupled in the p53-DNA complex, the bending specificity of the p53 system as well as complex stability could be fine-tuned by agents that affect DNA bending and twisting.

It has been shown (3) that DNA adducts of cisplatin and other platinum bifunctional compounds distort the conformation of DNA, including bending and changes in the twist angle. Thus, formation of the adducts by platinum compounds in the CDRE could affect its binding affinity to p53. No direct interaction between active wt p53 and platinum-modified DNA in cell-free media has yet been reported. Only binding of latent p53 protein, lacking sequence-specific DNA binding, to DNA modified by cisplatin has been reported (19). In the present work, we have used gel mobility shift assay methodology to investigate the binding affinity of the active human wt p53 system to the CDRE modified by either antitumor cisplatin or its clinically inefficient trans isomer in a cell-free medium. We have also examined binding of DNA lacking CDRE and modified by cisplatin or transplatin to reveal affinity of active wt p53 to platinated DNA containing no consensus nucleotide sequence. Thus, these studies could provide insight into the relative cytotoxicities of these two isomers, thereby potentially aiding in the rational design of new platinum drugs as well as illuminating aspects of the role of p53 in chemotherapy by its DNA binding activity. Active wt p53 binds to platinated DNA containing no consensus nucleotide sequence, but all contain two tandem consensus decamers, each a pentameric inverted repeat. Most consensus decamers, separated in the binding unit by 0–21 base pairs (bp), follow the consensus sequence pattern (17) PuPuPuPu(C/A)(T/A)GPpPyPy, where Pu and Py are purines and pyrimidines, respectively, and the vertical bar denotes the center of pseudodyad symmetry. Four molecules of the DNA-binding domain of p53 bind the response elements with high cooperativity. They also bend DNA. It has been suggested (18) that this bending is localized mostly at the two pentamer CA/TG junctions in the consensus DNA response element (CDRE) (by 25–28° at each junction) toward the major groove. Active wt p53 also over-twists the DNA response element by ~70°. This DNA twisting is uniformly distributed among the pentamers. It has also been suggested that due to many functions of p53 protein the demands for binding specificity and selectivity are necessarily extraordinary, which is accomplished through its tetrameric association with a repetitive binding site. Precise steric fit accommodated through both DNA bending and twisting appears extremely important in this binding site. Because DNA bending and twisting are coupled in the p53-DNA complex, the bending specificity of the p53 system as well as complex stability could be fine-tuned by agents that affect DNA bending and twisting.

It has been shown (3) that DNA adducts of cisplatin and other platinum bifunctional compounds distort the conformation of DNA, including bending and changes in the twist angle. Thus, formation of the adducts by platinum compounds in the CDRE could affect its binding affinity to p53. No direct interaction between active wt p53 and platinum-modified DNA in cell-free media has yet been reported. Only binding of latent p53 protein, lacking sequence-specific DNA binding, to DNA modified by cisplatin has been reported (19). In the present work, we have used gel mobility shift assay methodology to investigate the binding affinity of the active human wt p53 system to the CDRE modified by either antitumor cisplatin or its clinically inefficient trans isomer in a cell-free medium. We have also examined binding of DNA lacking CDRE and modified by cisplatin or transplatin to reveal affinity of active wt p53 to platinated DNA containing no consensus nucleotide sequence. Thus, these studies could provide insight into the relative cytotoxicities of these two isomers, thereby potentially aiding in the rational design of new platinum drugs as well as illuminating aspects of the role of p53 in chemotherapy by its DNA binding activity. Active wt p53 binds to platinated DNA containing no consensus nucleotide sequence, but all contain two tandem consensus decamers, each a pentameric inverted repeat. Most consensus decamers, separated in the binding unit by 0–21 base pairs (bp), follow the consensus sequence pattern (17) PuPuPuPu(C/A)(T/A)GPpPyPy, where Pu and Py are purines and pyrimidines, respectively, and the vertical bar denotes the center of pseudodyad symmetry. Four molecules of the DNA-binding domain of p53 bind the response elements with high cooperativity. They also bend DNA. It has been suggested (18) that this bending is localized mostly at the two pentamer CA/TG junctions in the consensus DNA response element (CDRE) (by 25–28° at each junction) toward the major groove. Active wt p53 also over-twists the DNA response element by ~70°. This DNA twisting is uniformly distributed among the pentamers. It has also been suggested that due to many functions of p53 protein the demands for binding specificity and selectivity are necessarily extraordinary, which is accomplished through its tetrameric association with a repetitive binding site. Precise steric fit accommodated through both DNA bending and twisting appears extremely important in this binding site. Because DNA bending and twisting are coupled in the p53-DNA complex, the bending specificity of the p53 system as well as complex stability could be fine-tuned by agents that affect DNA bending and twisting.
PvuII (blunt end-forming enzyme, which cuts twice within the pPGM1). This cleavage produced 474- and 2513-bp fragments containing and lacking CDRE, respectively. The two fragments were separated on the agarose gel, extracted, and purified. The 474-bp fragment was further globally modified by cisplatin or transplatin at \( r_p = 0.02-0.1 \), and the unmodified 2513-bp fragment was added as the nonspecific competitor. This mixture was incubated with various amounts of wt p53 (at molar ratios of p53 to 474-bp fragment in the range of 0–6) and analyzed using agarose gel electrophoresis. The incubation of the unmodified 474-bp fragment with increasing amounts of p53 resulted in the appearance of a new, more slowly migrating species, with a concomitant decrease of the intensity of the band corresponding to the 474-bp fragment incubated in the absence of p53 (shown for p53:474-bp fragment ratio of 0.95 in Fig. 2, lane 6). This result was in agreement with the previously published reports and demonstrated formation of a sequence-specific complex between DNA and p53 protein (30). Importantly, addition of DO-1 mAb (which maps to the N-terminal domain of p53) produced supershifted complexes that migrated still more slowly than the p53–474-bp complex (Fig. 2, lane 11), confirming the presence of p53 in the more slowly migrating species. In contrast, incubation of the 474-bp fragment modified by cisplatin at \( r_p = 0.02-0.06 \) with p53 (in the presence of the unmodified 2513-bp fragment) considerably reduced the yield of the species migrating more slowly in the agarose gel (shown for a p53 to 474-bp fragment ratio of 0.95 in Fig. 2, lanes 7–9). This result is consistent with the idea that cisplatin adducts efficiently reduce binding affinity of the CDRE to active p53. The same experiments were performed with the 474-bp fragment globally modified by transplatin. No reduction in the intensity of the band corresponding to the sequence-specific p53-DNA complex as a consequence of the modification by transplatin was noticed even at \( r_p \) as high as 0.1 (shown for \( r_p = 0.06 \) and a p53 to 474-bp fragment ratio of 0.95 in Fig. 2, lane 10). This result demonstrates the inefficiency of transplatin adducts to reduce the binding affinity of the CDRE to the active p53 protein.

Further studies were performed using a short (20 bp) oligodeoxyribonucleotide duplex, oligo-CDRE (Fig. 1) whose sequence follows the consensus sequence pattern (17). The duplex was globally modified by cisplatin or transplatin to \( r_p \) in the range of 0.02–0.06, and the unmodified PvuII fragment of pPGM1, which was 2513 bp long and contained no CDRE, was added as the nonspecific competitor. These mixtures were incubated with p53 at various p53 to duplex molar ratios (0.1–3) and analyzed using native PAGE (Fig. 3). Incubation of the unmodified oligo-CDRE with increasing amounts of p53 resulted in the appearance of the new, more slowly migrating species, with a concomitant decrease of the intensity of the band corresponding to the 20-bp duplex incubated in the absence of p53 (shown for a p53 to duplex ratio of 0.3 in Fig. 3, lane 2). This result confirmed the formation of the complex between oligo-CDRE and p53. In contrast, the incubation of oligo-CDRE-modified by cisplatin at \( r_p = 0.02-0.06 \) with p53 reduced the yield of the species migrating more slowly in the gel. For instance, the modification of oligo-CDRE by cisplatin at \( r_p = 0.05 \) already completely inhibited formation of the complex between this duplex and p53 (shown for a p53 to duplex molar ratio of 0.3 in Fig. 3, lane 4). It was also verified using PAGE under denaturing conditions that the modification of oligo-CDRE by cisplatin at \( r_p = 0.05 \) also afforded duplexes interstrand-cross-linked by this drug (data not shown). The quantitative evaluation (24, 25) of the radioactivities associated with the bands corresponding to interstrand-cross-linkedduplexes and duplexes containing no interstrand CL (24, 25) revealed ~20% of duplexes containing at least one interstrand CL of cisplatin.

Oligo-CDRE was also globally modified by transplatin and incubated with p53. No reduction in the intensity of the band corresponding to the p53-oligo-CDRE complex was noticed even at so high \( r_p \) as 0.05 (Fig. 3, lane 6), i.e. under conditions when cisplatin adducts inhibited formation of the complex between p53 and the duplex completely (Fig. 3, lane 4). Thus, these experiments confirmed that transplatin adducts do not affect the binding affinity of the CDRE to p53 protein.

**Binding of Active p53 Protein to Platinated DNA Lacking the Consensus Response Element**—We also investigated binding of p53 to the 448-bp fragment of the pBluescript II SK− plasmid lacking CDRE but modified by cisplatin or transplatin. The plasmid was cleaved by PvuII, which yielded the 448- and 2513-bp fragments. The longer fragment was identical to that produced by the PvuII cleavage of pPGM1, whereas the shorter fragment only differed from the shorter fragment produced by PvuII cleavage of pPGM1 by lacking CDRE. The 448-bp fragment was globally modified by cisplatin or transplatin at \( r_p = 0.01-0.08 \). After the 448-bp fragment was platinated, the unmodified 2513-bp fragment was added as the nonspecific competitor. These mixtures were incubated with p53 at various p53 to 448-bp fragment molar ratios (in the range of 0.5–6) and analyzed using agarose gel electrophoresis. Incubation of the unmodified PvuII fragments with increasing amounts of p53 resulted in no changes in the migration of these fragments, demonstrating no effect on formation of the complex between p53 and DNA lacking CDRE (shown for a p53 to 448-bp fragment ratio of 6 in Fig. 4, lane 6). On the other hand, a new
species migrating in the gel considerably more slowly was observed if the 448-bp fragment modified by cisplatin at $r_p = 0.01–0.08$ was analyzed (shown for a p53 to 448-bp fragment ratio of 6 in Fig. 4, lanes 7–9). This result demonstrated formation of the complex between p53 and DNA lacking CDRE and modified by cisplatin (30). Importantly, supershifted complexes were noticed as a consequence of addition of mAb DO-1 to the complex of p53 with the 448-bp fragment modified by cisplatin (Fig. 4, lane 11). The same experiments were performed with the 448-bp fragment globally modified by clinically ineffective transplatin. No more slowly migrating species as a consequence of the modification by transplatin was noticed at $r_p = 0.08$ and a p53 to DNA ratio of 6 (shown for $r_p = 0.08$ and a p53 to 448-bp fragment ratio of 6 in Fig. 4, lane 10). Thus, these results indicate that the binding affinity of p53 to DNA lacking CDRE is enhanced selectively by the modification by cisplatin and not by transplatin.

Cisplatin forms several types of adducts, which occur in DNA with a different frequency and differently distort the conformation of DNA (3). To determine which specific adduct of cisplatin represents a structural motif responsible for recognition of cisplatin-modified DNA lacking CDRE by p53, we prepared a series of 20-bp duplexes with blunt ends. The nucleotide sequences of these oligonucleotides were designed (Fig. 1) so that they did not follow the consensus sequence pattern (17), but they allowed us to prepare the duplexes containing a single site-specific adduct of cisplatin, such as 1,2-GG or 1,3-GTG intrastrand crosslink of cisplatin formed in DNA lacking CDRE. In the resulting complex. Extraordinary demands for this binding specificity and selectivity of p53 are closely related to its tetramer association with CDRE, in which a precise steric fit is extremely important. It has been suggested (18) that steric fit is accommodated through strongly correlated DNA bending localized at the two highly bendable CA/TG junctions and twisting uniformly distributed between the pentamers of the CDRE. The consensus sequences investigated in the present work contained several sites at which bifunctional adducts of cisplatin strongly distorting DNA conformation are formed. In particular, they contained the sites at which major intrastrand CLs between adjacent purines (at d(GG) or 5’-d(AG)) are formed. For instance, the structure of these adducts determined by NMR methods has revealed (31, 32) that these adducts induce the overall helix bend of 40°–78° toward the major groove, DNA unwinding of 25°–27°, severe perturbation of hydrogen bonding within the 5’-coordinated GC bp, and distortion extended over at least 4–5 bp at the site of the CL. Interestingly, the CDREs tested in the present work also contain the sites at which cisplatin forms less frequent intrastrand CLs.
Formation of these lesions in the CDREs investigated in the present work was confirmed by PAGE under denaturing conditions (see above). The interstrand CL, which is preferentially formed by cisplatin between opposite G residues in the 5′-GC/ 5′-GC sequence (24), induces several irregularities in the cross-linked base pairs and their immediately adjacent pairs (33). The cross-linked G residues are not paired with hydrogen bonds to the complementary cytosines, which are located outside the duplex and not stacked with other aromatic rings. All other base residues are paired, but distortion extends over at least 4 bp at the site of the CL. In addition, the cis-diaminodiamino platinum(II) bridge resides in the minor groove, and the double helix is locally reversed to a left-handed, Z-DNA-like form. This adduct induces the helix unwinding by −80° relative to B-DNA and also the bending of −40° of the helix axis at the cross-linked site toward the minor groove. Thus, cisplatin formed in the CDREs investigated in the present work bifunctional adducts, which strongly disturb its secondary structure. The result of these perturbations is that the precise steric fit required for the formation and stability of the tetrameric complex of p53 with the consensus nucleotide sequence cannot be attained, so that p53 does not bind to its CDRE.

We demonstrated in the present work that 1,2-intrastrand CLs of cisplatin formed in the CDRE reduce its binding affinity to p53, whereas the same adducts formed in the sequences that do not follow the CDRE pattern afford DNA enhanced binding affinity to p53. Hence, the CDRE should have some intrinsic specific feature absent in the usual B-DNA that precludes p53 from binding to CDRE after its modification by cisplatin. It has been shown (18) that CDREs are already intrinsically curved in the flexible CATG tetramers, with the directionality close to that in the p53-DNA complexes. We suggest that unique alterations induced in the consensus sequence simultaneously by 1,2-intrastrand CLs of cisplatin and the intrinsic curvature of the CDRE are structural factors responsible for the reduced affinity of p53 to its consensus sequence modified by cisplatin.

Clinically ineffective transplatin also forms various types of adducts in DNA. It forms mainly monofunctional adducts at G residues and some amount of interstrand CLs between complementary G and C residues (6, 7, 25). Monofunctional adducts of transplatin (and cisplatin) affect DNA conformation only slightly, without bending (20), and DNA unwinding is very small (−6°) (34). In addition, the conformational alterations induced by the interstrand CL of transplatin are much less severe than those induced by the CLs of cisplatin (33, 35). The duplex is slightly distorted on both sides of the CL, but all bases are still paired and hydrogen-bonded. The CL of transplatin only unwinds the double helix by −12° and induces a slight, flexible bending of −20° of its axis toward the minor groove. We conclude that these relatively subtle structural perturbations induced by transplatin in the CDRE have no substantial effect on the formation of the tetrameric complex of p53 with the CDRE.

1,2-Intrastrand Cross-links of Cisplatin Formed in DNA Lacking the Consensus Nucleotide Sequence Increase DNA Binding Affinity to Active wt p53 Protein—The results of the present work also demonstrate enhancement of the binding affinity of p53 to DNA lacking CDRE due to its modification by cisplatin. However, this binding affinity of p53 is considerably lower (roughly by one order of magnitude) than that to the unplatinated CDRE. Importantly, no change of the binding affinity to p53 of DNA lacking CDRE is observed due to its modification by clinically ineffective transplatin; thus this enhancement is specific for cisplatin-modified DNA. Also importantly, among DNA adducts of cisplatin only the 1,2-intrastrand CLs are responsible for this increase in DNA binding to p53. Hence, 1,2-intrastrand CLs distort DNA lacking the CDRE in a specific way, producing a structural motif recognized by p53. We propose that directional and stable bending of DNA lacking the CDRE toward its major groove due to formation of the 1,2-intrastrand CL of cisplatin affords a structural element exhibiting this specific affinity to p53. In this way a stable flexure of DNA by 1,2-intrastrand CL provides an opportunity for more stable contacts between p53 and DNA lacking CDRE. In other words, the lesions such as 1,2-intrastrand CLs that efficiently induce the directional and fixed bend in DNA toward the major groove, thus providing a stable pre-bent site on DNA to p53, serve as a structural motif for recognition of DNA lacking CDRE by p53.

The observation that other minor bifunctional DNA adducts formed by cisplatin in usual B-DNA lacking CDRE, which also bend and unwind DNA, are not recognized by p53 deserves further discussion. The intrastrand CL formed by cisplatin between two G residues separated by a third base also bends the helix axis toward the major groove by −30° (36) and locally unwinds DNA by −19° (26). However, another important feature of the conformational alteration induced by this lesion is that DNA is locally denatured and flexible at the site of the adduct (36), the structural feature much less pronounced in the 1,2-intrastrand adduct. Furthermore, interstrand CLs of cisplatin also distort DNA distinctly differently and more severely than intrastrand adducts (see above). These significant structural differences between the latter minor adducts and the major 1,2-intrastrand CLs suggest that their recognition and binding by active p53 will be different and restricted to the structural features identical or similar to those induced in DNA by the 1,2-intrastrand adduct of cisplatin. Consistent with this idea is the observation that there are also significant structural differences between 1,2-intrastrand CLs of cisplatin and the adducts formed on DNA by transplatin (see above), which do not form the lesions recognized by p53 on DNA lacking CDREs. Thus, the data base on the structures of cisplatin and transplatin adducts reveals their propensity to distort DNA in very different ways. The distinctive structural features of 1,2-intrastrand CLs of cisplatin suggest a unique role for this adduct in the enhancement of the binding of p53 to platinated DNA segments lacking CDRE, which is, however, weaker than the binding of p53 to unplatinated CDRE.

Biological Implications—There is substantial evidence suggesting that p53 plays a central role in the cellular response to DNA damage. It is also clear that p53 function may only be one of many factors that modulate cisplatin sensitivity, and the effects may be different for various cell types. In addition, sequence-specific DNA binding is one of the key biochemical activities responsible for much of the biological function of p53. Hence, the observation described in the present work demonstrating that cisplatin adducts formed in the CDRE reduce its binding affinity to active p53 may affect these key biochemical activities. Because the affinity of p53 to the CDRE is not affected by the adducts of clinically ineffective transplatin, we suggest that the reduced affinity of the active p53 protein to the CDRE due to its modification by cisplatin is relevant to the biological activity of this drug.

A possible intriguing scenario for response to cellular exposure to cisplatin is also that associated with another important result of the present work demonstrating a relatively weak but significant increase in the binding affinity of p53 to major adducts of cisplatin-1,2-intrastrand CLs formed in the parts of DNA lacking CDRE. This increase in the binding affinity of p53 is specific for DNA modified by cisplatin and does not occur if DNA is modified by clinically ineffective transplatin. This result is consistent with the view that the mechanism of antitumor activity of cisplatin may also be associated with its effi-
ciency to promote binding activity of p53 in the segments of DNA lacking CDRE. We suggest that 1,2-intrastrand CLs formed by cisplatin in DNA segments lacking CDRE may be sufficient to hijack p53 protein, keeping it away from its natural targets. Thus, the resultant complexes may divert p53 from its natural functions and/or may protect the cisplatin damage from its recognition by other cellular components. Alternatively, it has been shown (37) that p53 enhances binding of DNA modified by cisplatin to chromosomal high mobility group (HMG) 1 protein. HMG domain proteins are known to specifically bind 1,2-intrastrand CLs of cisplatin and thus mediate antitumor effects of this drug (3). 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 shielding cisplatin-DNA adducts from excision repair or titrating away these proteins from their transcriptional regulatory function have been suggested as clues to how these proteins are involved in the antitumor activity (9). It is therefore possible that p53 and HMG1 protein encounter each other at the sites of 1,2-intrastrand adducts of cisplatin (formed outside the CDRE). This interaction could further promote keeping p53 away from its natural target and/or promote the binding affinity of HMG1 protein to cisplatin-modified DNA, with the consequences for the antitumor effect of cisplatin mentioned above. More detailed information about cellular consequences of interactions of platinated DNA and p53 protein is required before any definite conclusions can be drawn about how these interactions contribute to the mechanism of the biological activity of cisplatin.

Acknowledgment—We thank Dr. B. Vojtesek for critical reading of the manuscript and helpful discussions.

REFERENCES

1. Weng, E., and Giandomenico, C. M. (1999) Chem. Rev. 99, 2451–2466
2. O’Dwyer, P. J., Stevenson, J. P., and Johnson, S. W. (1999) in Cisplatin: Chemistry and Biochemistry of a Leading Anticancer Drug (Lippert, B., ed) pp. 31–72, Verlag Helvetica Chimica Acta, Zurich
3. Jamieson, E. R., and Lippard, S. J. (1999) in Cisplatin: Chemistry and Biochemistry of a Leading Anticancer Drug (Lippert, B., ed) pp. 31–72, Verlag Helvetica Chimica Acta, Zurich
4. Jamieson, E. R., and Lippard, S. J. (1999) in Cisplatin: Chemistry and Biochemistry of a Leading Anticancer Drug (Lippert, B., ed) pp. 31–72, Verlag Helvetica Chimica Acta, Zurich
5. Leng, M., Schwartz, A., and Giraud-Panis, M. J. (2000) in Platinum-Based Drugs in Cancer Therapy (Kelland, L. R., and Farrell, N. P., eds) pp. 63–85, Humana Press Inc., Totowa, NJ
6. Leng, M., Locker, D., Giraud-Panis, M. J., Schwartz, A., Intini, F. P., Natile, G., Pisano, C., Boccarelli, A., Giordano, D., and Colucia, M. (2000) Mol. Pharmacol. 58, 1525–1535
7. Eastman, A. (1999) in Cisplatin: Chemistry and Biochemistry of a Leading Anticancer Drug (Lippert, B., ed) pp. 111–134, Verlag Helvetica Chimica Acta, Zurich
8. Eastman, A. (1999) in Cisplatin: Chemistry and Biochemistry of a Leading Anticancer Drug (Lippert, B., ed) pp. 73–110, Verlag Helvetica Chimica Acta, Zurich
9. Jordan, P., and Carmo-Fonseca, M. (2000) Cell. Mol. Life Sci. 57, 1229–1235
10. Burne, T. F., and El-Deiry, W. S. (1999) J. Cell. Physiol. 181, 231–239
11. Bates, S., and Vousden, K. H. (1999) Cell. Mol. Life Sci. 55, 26–37
12. Janus, F., Albrechtus, N., Durnreiter, I., Wiesmuller, L., Groese, F., and Doppert, W. (1999) Cell. Mol. Life Sci. 55, 12–27
13. May, P., and May, E. (1999) Oncogene 18, 7621–7636
14. Zamble, D. B., Tyler, J., and Lippard, S. J. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 6163–6168
15. O’Connor, P. M., Jackman, J., Bae, I., Myers, T. G., Fan, S., Mutoh, M., Scudiero, D. A., Monks, A., Sausville, E. A., and Weinstein, J. N. (1997) Cancer Res. 57, 4285–4300
16. El-Deiry, W. S., Kern, S. E., Pietenpol, J. A., Kinzler, K. W., and Vogelstein, B. (1992) Nat. Genet. 1, 45–49
17. Nagaslu, A. K., Zhurkin, V. B., Durell, S. R., Jernigan, R. L., Appella, E., and Harrington, R. E. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 1875–1880
18. Wetzel, C. C., and Berberich, S. J. (1998) Oncology Res. 10, 151–161
19. Brabec, V., Reedijk, J., and Leng, M. (1992) Biochemistry 31, 12397–12402
20. Brabec, V., Reedijk, J., and Leng, M. (1992) Biochemistry 31, 12397–12402
21. Brabec, V., Reedijk, J., and Leng, M. (1992) Biochemistry 31, 12397–12402
22. Hupp, T. R., and Lane, D. P. (1995) J. Biol. Chem. 270, 18165–18174
23. Kim, S. D., Vrana, O., Kleinwächter, V., Niki, K., and Brabec, V. (1999) Anal. Lett. 33, 1505–1518
24. Lemaire, M. A., Schwartz, A., Rahmouni, A. R., and Leng, M. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 1982–1985
25. Brabec, V., and Leng, M. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 5345–5349
26. Bellon, S. F., Coleman, J. H., and Lippard, S. J. (1991) Biochemistry 30, 8026–8035
27. Kasparkova, J., and Brabec, V. (1995) Biochemistry 34, 12379–12387
28. Brabec, V., Nplechova, K., Kasparkova, J., and Farrel, N. (2000) J. Biol. Inorg. Chem. 5, 364–368
29. Vojtesek, B., Bartek, J., Midgley, C. A., and Lane, D. P. (1992) J. Immunol. Methods 151, 237–244
30. Mazur, S. J., Sakaguchi, K., Appella, E., Wang, X. W., Harris, C. C., and Bohr, V. A. (1999) J. Mol. Biol. 292, 241–249
31. Yang, D. Z., van Boom, S. S. G. E., van Boom, S. H., and Wang, A. H. J. (1999) Biochemistry 34, 12921–12929
32. Gelasco, A., and Lippard, S. J. (1998) Biochemistry 37, 9230–9239
33. Brabec, V. (2000) in Platinum-Based Drugs in Cancer Therapy (Kelland, L. R., and Farrell, N. P., eds) pp. 37–61, Humana Press Inc., Totowa, NJ
34. Keck, M. V., and Lippard, S. J. (1992) J. Am. Chem. Soc. 114, 3386–3390
35. Brabec, V., Sip, M., and Leng, M. (1993) Biochemistry 32, 11676–11681
36. Teubner, J. M., Bauer, C., Wang, A. H. J., and Reedijk, J. (1999) Biochemistry 38, 12295–12312
37. Imamura, T., Izumi, H., Nagatani, G., Ise, T., Nomoto, M., Iwamoto, Y., and Kohno, K. (2001) J. Biol. Chem. 276, 7534–7540
Different Recognition of DNA Modified by Antitumor Cisplatin and Its Clinically Ineffective trans Isomer by Tumor Suppressor Protein p53
Jana Kasparkova, Sarka Pospisilova and Viktor Brabec

J. Biol. Chem. 2001, 276:16064-16069. doi: 10.1074/jbc.M101224200 originally published online March 6, 2001

Access the most updated version of this article at doi: 10.1074/jbc.M101224200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 31 references, 9 of which can be accessed free at http://www.jbc.org/content/276/19/16064.full.html#ref-list-1