Trifunctional Dinuclear Platinum Complexes as DNA−Protein Cross-Linking Agents†

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ABSTRACT: The trifunctional dinuclear platinum compounds 1,2/c,c [{*cis*-$PtCl(NH_3)_2$}μ-$H_2N(CH_2)_6NH_2$-{*cis*-$PtCl_2(NH_3)$}]$^+$ and 1,2/t,c [{*trans*-$PtCl(NH_3)_2$}μ-$H_2N(CH_2)_6NH_2${*cis*-$PtCl_2(NH_3)$}]$^+$ contain a monofunctional platinum coordination sphere linked to a *cis*-[$PtCl_2$(amine)$_2$] moiety. The compounds have been examined for their DNA binding and ability to induce covalent ternary DNA−protein cross-links. Comparison was made with representative bifunctional dinuclear platinum compounds [{$PtCl(NH_3)_2$}$_2\mu$-$H_2N(CH_2)_nNH_2$]$^{2+}$. DNA modified by the trifunctional compounds is able to bind and cross-link *Bam*HI, a sequence-specific DNA-binding protein that recognizes the palindromic sequence GGATCC and also very efficiently binds and cross-links SP1, a sequence-specific Zn finger protein that induces a bend in the DNA upon binding. Two representative nonsequence-specific DNA-binding proteins, the Klenow fragment from DNA polymerase I and Klenow exonuclease minus (which has been mutated to remove the 3′−5′ proofreading domain), both bind modified DNA and effectively cross-link to the DNA. Data from circular dichroism, inhibition of ethidium bromide fluorescence, interstrand cross-linking and unwinding assays are all consistent with (Pt,Pt) interstrand cross-links as the dominant lesion of trifunctional compounds and the most likely structure to form the ternary DNA−protein cross-links. In vitro transcription of RNA is inhibited by the platinum compounds and indicate G residues as primary binding sites. Binding to calf thymus DNA as assessed by differential pulse polarography is rapid and essentially quantitative. An increase in melting temperature of CT DNA adducted by the platinum compounds is observed at low salt concentrations but at high salt, modification results in a decrease of t_m. In summary, the trifunctional agents may find use as protein-targeting drugs and as probes for conformational effects on DNA−protein interactions.

Polynuclear platinum complexes represent a distinct structural class of anti-cancer agents. Within this general class, the presence of at least two platinum coordination units allows for considerable structural diversity where DNA binding may in principle be tetrafunctional, trifunctional, or bifunctional (*1*). The predominant DNA lesions of dinuclear platinum complexes are long-range inter- and intrastrand cross-links (*2*−*4*). Since, by definition, a DNA−DNA cross-link only requires two substitution sites, multifunctional dinuclear coordination compounds are also capable of the formation of ternary DNA−protein cross-links (*5*). The tetrafunctional *cis*-[{$Pt(NH_3)Cl_2$}$_2\mu$-$H_2N(CH_2)_4NH_2$] (2,2/c,c) has been shown to effect such chemistry, as demonstrated by the cross-linking of components of the bacterial Uvr repair system to DNA−DNA *interstrand* adducts induced by the complex (*6*). Heterodinuclear [Pt,Ru] compounds are also capable of inducing these ternary DNA−protein cross-links (*6*, *7*).

DNA−protein cross-linking agents may have a host of possible applications, including identification of contact sites, isolation of weakly bound proteins within a multiprotein complex, and representing an attractive target for chemotherapeutic intervention. For the latter application, it is therefore desirable to explore the potential selectivity of such interactions in terms of both DNA lesions and protein target. One disadvantage of the 2,2/c,c system previously reported is that cisplatin-like DNA adducts may also form through the bifunctional reaction of only one coordination sphere, even though (Pt,Pt) interstrand cross-links are dominant (*5*, *8*). Since it is clear from studies on bifunctional dinuclear and trinuclear complexes that protein recognition is distinctly different between (Pt,Pt) interstrand cross-links and that the *cis*-DDP 1,2 intrastrand adduct (*9*−*11*), more selective interstrand cross-linking agents capable of forming DNA−protein adducts are desirable.

Trifunctional dinuclear platinum compounds 1,2/c,c [{*cis*-$PtCl(NH_3)_2$}$H_2N(CH_2)_6NH_2${*cis*-$PtCl_2(NH_3)$}]$^+$ and 1,2/t,c [{*trans*-$PtCl(NH_3)_2$}$H_2N(CH_2)_6NH_2${*cis*-$PtCl_2(NH_3)$}]$^+$ (Fig-

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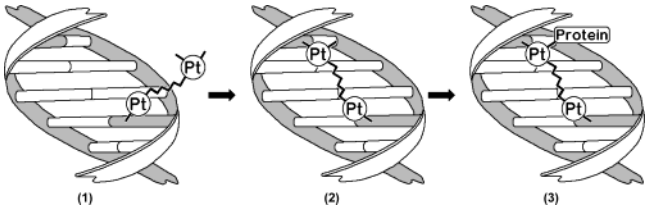
ure 1) have been recently reported where one cisplatin group is linked to a monofunctional coordination sphere (*12*, *13*). Model studies indicated some preference for binding of the mononucleotide 5′-guanosine monophosphate (5′-GMP)[1] to the monofunctional end of the molecule. Extension of this binding preference to DNA would thus automatically set up an interstrand cross-link in the second reaction step. The third Pt−Cl bond is then available for protein binding in a specific stepwise manner:



It was therefore of interest to examine the DNA binding and ternary DNA−protein cross-link formation of these trifunctional compounds. This paper reports on the reactivity of these trifunctional dinuclear platinum compounds to random-sequence DNA, the conformational changes induced upon DNA binding, sequence specificity, and DNA−DNA interstrand cross-linking efficency and on the ability of the DNA adducts to form ternary complexes with both sequence-specific (*Bam*HI and SP1, a Zn finger protein) and non-sequence-specific (the Klenow fragment from DNA polymerase I and Klenow exonuclease minus, which has been mutated to remove the 3′−5′ proofreading domain) DNA-binding proteins.

MATERIALS AND METHODS

Starting Materials. Cisplatin (Figure 1) was obtained from Sigma-Aldrich sro (Prague, Czech Republic). The dinuclear trifunctional complexes 1,2/t,c and 1,2/c,c (Figure 1) were prepared and characterized as described previously (*12*, *13*). Chlorodiethylenetriamineplatinum(II) chloride, [PtCl(dien)]-Cl, was kindly provided by Dr. Giovanni Natile (University of Bari, Italy). Stock solutions of platinum compounds for the physical studies were prepared at the concentration of 5×10^{-4} M in 10 mM $NaClO_4$ and stored at 4 °C in the dark. Calf thymus (CT) DNA (42% G + C, mean molecular mass ca. 2×10^7) was also prepared and characterized as described previously (*14*, *15*). All plasmids were isolated according to standard procedures and banded twice in CsCl/EtBr equilibrium density gradients. All proteins for ternary reactions were commercially available.

Platination Reactions. CT and plasmid pSP73KB and pUC19 DNAs were incubated with platinum complex in 10 mM $NaClO_4$ at 37 °C for 48 h in the dark. The r_b value was determined by flameless atomic absorption spectrophotometry (FAAS) or by differential pulse polarography (DPP) (*16*). For example, CT DNA at a concentration of 0.32 mg/mL was incubated with platinum complex at an initial 0.01 r_i in 10 mM $NaClO_4$ at 37 °C. At various time intervals, an aliquot of the reaction mixture was withdrawn and assayed by DPP for platinum not bound to DNA. The amount of platinum bound to DNA (r_b) was calculated by subtracting the amount of free (unbound) platinum from the total amount of platinum present in the reaction. No changes in the pH of the reaction mixture containing DNA and either 1,2/t,c or 1,2/c,c were measured within 48 h after mixing DNA with the platinum complex.

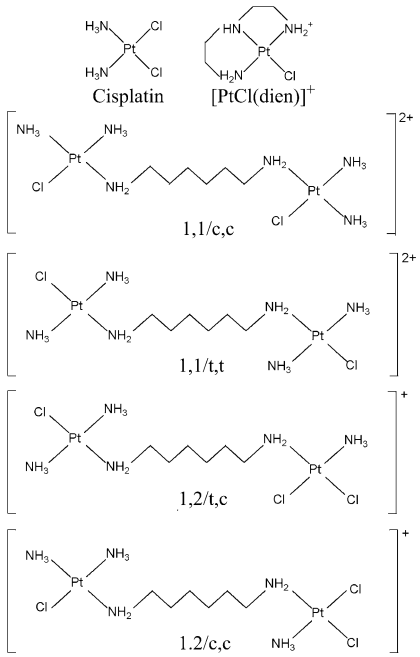


FIGURE 1: Structures of platinum compounds used in the present work.

In the case of the 5′-end-labeled *Eco*RI−*Hin*dIII fragments from pGem and pSP (5 nM), increasing amounts of platinum compound in TE (10 mM Tris-HCl, 0.1 mM EDTA, pH 8) were mixed with DNA and incubated at 37 °C for 1 h. Reactions were terminated by the addition of 400 mM NaCl, and the adducted DNA was purified by Sephadex G-25 spin columns to remove any unbound drug, followed by ethanol precipitation. Samples were resuspended in TE and analyzed by gel electrophoresis under denaturing conditions (10% PAGE, 8 M urea, 50 °C). Quantification of free and adducted DNA was determined with a Molecular Dynamics PhosphorImager, and Pt content was confirmed by FAAS.

DNA Melting. The melting curves of CT DNAs were recorded by measuring the absorbance at 260 nm. The melting curves of unplatinated or platinated DNA were recorded after Tris-HCl/EDTA buffer and $NaClO_4$ were added so that the resulting media contained 0.01 or 0.2 M $NaClO_4$ with 1 mM Tris-HCl/0.1 mM EDTA, pH 7.4. The value of the melting temperature (t_m) was determined as the temperature corresponding to a maximum on the first-derivation profile of the melting curves. The t_m values could be thus determined with an accuracy of ±0.3 °C.

Fluorescence Measurements. These measurements were performed on a Shimadzu RF 40 spectrofluorophotometer using a 1-cm quartz cell. Fluorescence measurements of DNA modified by platinum in the presence of EtBr were performed at an excitation wavelength of 546 nm, and the emitted fluorescence was analyzed at 590 nm. The fluores-

[1] Abbreviations: CD, circular dichroism; EtBr, ethidium bromide; CT, calf thymus; 5′-GMP, 5′-guanosine monophosphate; [PtCl(dien)]-Cl, chlorodiethylenetriamineplatinum(II) chloride; t_m, melting temperature of DNA; r_i is defined as the molar ratio of free platinum complex to nucleotide phosphates at the onset of incubation with DNA; r_b is the number of Pt complexes bound per nucleotide residue; CL, cross-link; DTT, dithiothreitol; FAAS, flame absorption atomic spectroscopy; HSQC, heteronuclear single quantum coherence; HMG, high mobility group; G, guanine.

Table 1: Summary of DNA Binding of Dinuclear Trifunctional Platinum Complexes and Comparison with DNA Binding of Dinuclear Bifunctional Complexes, Mononuclear Cisplatin, and [PtCl(dien)Cl]Cl

	1,2/t,c	1,2/c,c	1,1/t,t	1,1/c,c	cisplatin	[PtCl(dien)Cl]Cl
DNA binding ($t_{1/2}$)	20 min[a]	20 min[a]	40 min[b]	40 min[b]	~120 min[c]	~120 min[d]
nucleotide preference	G[a]	G[a]	G[b]	G[e]	G, A[f]	G[d]
decrease of EtBr fluorescence	strong[a]	strong[a]	strong[b]	strong[e]	medium[b,e]	weak[e]
% interstrand CL/adduct (after 48 h)	82[a]	85[a]	70−90[b,g]	87−95[e,g]	6[h]	no
unwinding angle/adduct	10°[a]	10°[a]	10°[g]	12°[g]	13°[i,j]	6°[i]
CD at ~280 nm	increase[a]	increase[a]	increase[b]	increase[e]	increase[k]	decrease[k]
melting temperature						
high ionic strength	decrease[a]	decrease[a]	decrease[b]	nd[n]	decrease[l]	decrease[l]
low ionic strength	increase[a]	increase[a]	increase[b]	increase[e]	decrease[l]	increase[l]
DNA−protein cross-linking	yes	yes	MF[m] only	nd	MF only	no

[a] This work. [b] Ref *24*. [c] Ref *63*. [d] Ref *64*. [e] Ref *23*. [f] Ref *65*. [g] Ref *37*. [h] Ref *20*. [i] Ref *22*. [j] Ref *35*. [k] Ref *26*. [l] Ref *25*. [m] Monofunctional adducts only. [n] nd, not determined.

cence intensity was measured at 25 °C in 0.4 M NaCl to avoid secondary binding of EtBr to DNA (*17*, *18*). The concentrations were 0.01 mg/mL for DNA and 0.04 mg/mL for EtBr, which corresponded to the saturation of all intercalation sites of EtBr in DNA (*18*).

DNA Transcription by RNA Polymerases in Vitro. Transcription of the (*Nde*I/*Hpa*I) restriction fragment of pSP73KB DNA with T7 RNA polymerase and electrophoretic analysis of transcripts was performed according to the protocols recommended by Promega (Promega Protocols and Applications, 43-46 (1989/1990) and previously described in detail (*19*, *20*).

DNA Interstrand Cross-Linking. Platinum complexes at varying concentrations were incubated with 2 μg of pSP73 DNA linearized by *Eco*RI. The platinated samples were precipitated by ethanol and analyzed for DNA interstrand cross-links by previously published procedures (*19*, *21*). The linear duplexes were first 3′-end labeled by means of Klenow fragment of DNA polymerase I in the presence of [α-^{32}P]-dATP. The samples were deproteinized by phenol and precipitated by ethanol, and the pellet was dissolved in 18 μL of a solution containing 30 mM NaOH, 1 mM EDTA, 6.6% sucrose, and 0.04% bromophenol blue. The amount of interstrand CLs was analyzed by electrophoresis under denaturing conditions on alkaline agarose gel (1%). After the electrophoresis was completed, the intensities of the bands corresponding to single strands of DNA and interstrand cross-linked duplex were quantified by means of a Molecular Dynamics PhosphorImager (Storm 860 system with ImageQuant software).

Unwinding of Negatively Supercoiled DNA. Unwinding of closed circular supercoiled pUC19 plasmid DNA was assayed by an agarose gel mobility shift assay (*22*). The unwinding angle (Φ) induced per platinum−DNA adduct was calculated upon the determination of the r_b value at which the complete transformation of the supercoiled to relaxed form of the plasmid was attained. Samples of pSP73 plasmid were incubated with 1,2/t,c or 1,2/c,c at 37 °C in the dark for 48 h. All samples were precipitated by ethanol and redissolved in the TBE (Tris−borate/EDTA) buffer. An aliquot of the precipitated sample was subjected to electrophoresis on 1% agarose gels running at 25 °C in the dark with TBE buffer and the voltage set at 30 V. The gels were then stained with EtBr, followed by photography on Polaroid 667 film with transilluminator. The other aliquot was used for the determination of r_b values by FAAS.

Preparation of Proteins. To remove DTT from the commercially available proteins *Bam*HI, SP1, Klenow exonuclease minus (exo$^-$), and Klenow, the manufacturer's storage buffers were exchanged using microcon concentrators. The final buffer composition for *Bam*HI and Klenow was 10 mM Tris, pH 8, 0.5 mM EDTA, 100 μg/mL BSA, and 50% glycerol. The final buffer composition for Klenow exo$^-$ was supplemented with 10 mM $MgSO_4$, and the final buffer composition of SP1 was supplemented with 10 mM $MgSO_4$ and 2 mM tris-(2-carboxyethyl)phosphine (TCEP).

Ternary DNA−Protein Reactions. Platinated DNA (1 nM) was incubated with the various proteins at 25 °C for 1 h in the appropriate buffer: 10 mM Tris, pH 8, 10 mM EDTA, 0.1 μg of BSA, and 0.8% glycerol (*Bam*HI and Klenow); 10 mM Tris, pH 8, 10 mM EDTA, 0.1 μg of BSA, 0.8% glycerol, and 2 mM $MgSO_4$ (SP1 and Klenow exo$^-$). The reactions were divided in two and analyzed by native gel electrophoresis (4.5% 0.5×TBE PAGE) and 5%/10% SDS−PAGE (samples were loaded in cracking buffer without DTT). Quantification of free and adducted DNA was determined with a Molecular Dynamics PhosphorImager.

In Situ Ternary Reactions. In these studies, uncomplexed duplexes were treated with indicated proteins in 10 mM Tris-HCl [pH 8, 0.1 mM EDTA, 0.8% glycerol, and 0.1 μg of BSA (supplemented with 2 mM $MgSO_4$ for Klenow exo$^-$ and SP1 reactions)] at 25 °C for 15 min. Platinum complex (10 μM final concentration) was added, and the reaction was incubated at 25 °C for 1 h and analyzed as previously described.

RESULTS

Physical Measurements. (*a*) *CT DNA Binding.* The amount of platinum coordinated to DNA increased with time, and after approximately 6 h the complex was quantitatively bound. The half-time ($t_{1/2}$) of this binding reaction was ~20 min. The binding of the dinuclear trifunctional platinum complexes is thus faster than that of the dinuclear bifunctional compounds 1,1/t,t and 1,1/c,c [$t_{1/2}$ ~40 min (*23*), Table 1]. The binding is also faster than the monfunctional [PtCl(dien)]$^+$, which carries the same formal charge. This may be attributed to the availability of the two Pt coordination spheres for DNA binding. The rapid and essentially quantitative binding of 1,2/t,c and 1,2/c,c facilitates sample analysis. The binding experiments indicate that such platination reactions resulted in the coordination of all molecules of the platinum complexes, making it possible to prepare

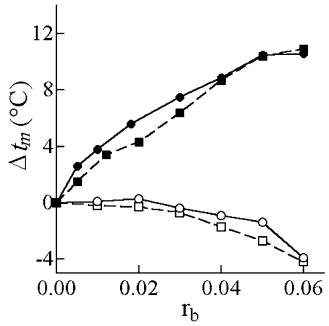


FIGURE 2: Plots of Δt_m values of CT DNA modified by 1,2/t,c (squares) or 1,2/c,c (circles) on r_b. The t_m values were measured in media containing 0.01M (solid symbols) or 0.2 M (open symbols) $NaClO_4$; the solutions also contained 1 mM Tris-HCl and 0.1 mM EDTA, pH 7.4 (Δt_m is defined as the difference between the t_m values of platinated and nonmodified DNAs).

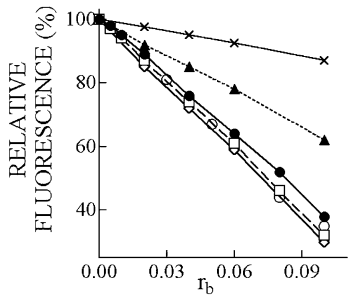


FIGURE 3: Dependences of the EtBr fluorescence on r_b for DNA modified by various platinum complexes in 10 mM $NaClO_4$ at 37 °C for 48 h. 1,2/t,c (□), 1,2/c,c (●), 1,1/t,t (◇), 1,1/c,c (o), cisplatin (▲), and [PtCl(dien)]Cl (×).

easily and precisely DNA samples modified at a preselected value of r_b.

(*b*) *DNA Melting.* CT DNA was modified by 1,2/t,c or 1,2/c,c to various r_b (0−0.06) in 10 mM $NaClO_4$. Salt concentration was then further adjusted by addition of $NaClO_4$ to the values of 0.01 or 0.2 M. The effect on t_m is dependent both on the amount of platinum bound and the salt concentration. At low concentrations of $NaClO_4$ (0.01 M), an increase of t_m is observed even at relatively high levels of the modification of DNA by 1,2/t,c or 1,2/c,c (at $r_b = 0.06$, Δt_m was ∼12 °C) (Figure 2). At high salt concentrations of 0.2 M, the modification resulted in a decrease of t_m that became more pronounced with increasing r_b value (Figure 2). Thus, the dinuclear trifunctional compounds 1,2/t,t or 1,2/c,c affected t_m qualitatively in the same way as dinuclear bifunctional compounds 1,1/t,t or 1,1/c,c (*23*, *24*). This behavior is in marked contrast to cisplatin, where the modification of DNA results in a decrease of t_m if DNA melting is measured in salt concentrations from 0.01 to 0.2 M (*25*).

Previously, three factors have been invoked to account for the thermal stability of DNA modified by platinum(II) complexes: stabilizing effects of the positive charge on the platinum(II) moiety and of DNA interstrand CLs and a destabilizing effect of conformational distortions induced in DNA by platinum coordination resulting in various types of adducts (*25*). The dependence of transition melting temperature on ionic strength was explained by competing electrostatic effects as salt concentration was varied. Under the incubation conditions, we expect that all bifunctional platinum compounds produced a range of CLs and that the observed change in melting temperature will reflect the relative proportion and contribution of the two limiting factors. Inherently, we predict conformational alterations due to intrastrand cross-linking to destabilize the helix, as has been consistently observed in studies with cisplatin. In contrast, interstrand cross-linking is also predicted to stabilize the helix by preventing strand dissociation. At low ionic strength, it is reasonable to conclude that the increases in t_m are caused by the high percentage of interstrand CLs formed by all dinuclear compounds and by positive charges on platinum moieties. On the other hand, the observation that high salt appears to result in destabilization, even in the presence of interstrand CLs seems to be a consequence of conformational changes induced by the platinum adducts, which then dominate over the combination of "stabilizing" effects. At high salt concentration, the stabilizing effects are reduced since electrostatic effects of the platinum compounds are apparently lowered with increasing concentration of Na^+ counterions. Thus, the solution behavior of the DNA adducts of dinuclear trifunctional compounds tested in the present work appears very similar to that of the DNA adducts of dinuclear bifunctional compounds and support the idea that the DNA binding mode of the dinuclear bifunctional and trifunctional platinum compounds are similar.

Conformational Alterations Studied by CD. CD spectra of CT DNA modified by 1,2/t,c and 1,2/c,c complexes were recorded for various r_b values, and their shape (not shown) was essentially identical to those recorded under the same conditions for CT DNA modified by dinuclear bifunctional compounds 1,1/t,t and 1,1/c,c (*23*, *24*). The intensity of the positive CD band of DNA at about 280 nm (which has been used to evaluate conformational alterations induced in DNA by various agents including platinum compounds (*26*) is increased with the growing level of the DNA modification up to $r_b = 0.05$ (summarized in Table 1). A further increase of the modification resulted in gradual decrease of the intensity of this band.

Characterization of DNA Adducts by EtBr Fluorescence. EtBr as a fluorescent probe has been used to characterize perturbations induced in DNA by bifunctional adducts of several mononuclear and polynuclear platinum compounds (*23*, *24*, *27*−*29*). Double-helical DNA was first modified to an r_b in the range between 0 and 0.1 by cisplatin, monofunctional [PtCl(dien)]Cl, dinuclear bifunctional 1,1/t,t or 1,1/c,c, and dinuclear trifunctional 1,2/t,c or 1,2/c,c. Modification of DNA by all platinum complexes resulted in a decrease of EtBr fluorescence (Figure 3). The decrease caused by the adducts of all dinuclear platinum complexes was similar and markedly more pronounced than that induced by the DNA adducts of cisplatin at equivalent r_b. Modification of DNA by monofunctional platinum complexes results in only a slight decrease of EtBr fluorescence intensity as compared with the control DNA−EtBr complex (*23*, *24*, *27*−*29*). The structures of dinuclear bifunctional DNA adducts arise from two monofunctional substitutions on the polynucleotide. Comparison with [PtCl(dien)]Cl suggests that the conformational distortion induced in DNA by the dinuclear trifunctional adducts is much more delocalized and extends over considerably more base pairs around the platination sites and is not simply the "sum" of two monofunctional lesions. Thus, these results are also consistent with the formation of

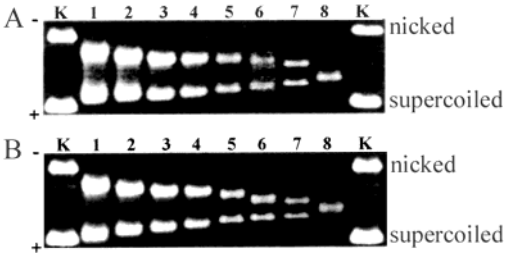


FIGURE 4: Unwinding of supercoiled pUC19 plasmid DNA by 1,2/c,c (A) and 1,2/t,c (B) complexes. The top bands correspond to the form of nicked plasmid and the bottom bands correspond to closed, negatively supercoiled plasmid. The plasmid was incubated with the r_b values of 0 (control) (lanes K), 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.10 (lanes 1−8, respectively).

long-range intra- or interstrand CLs of dinuclear trifunctional platinum complexes.

In Vitro Transcription of DNA-Containing Platinum Adducts. In vitro RNA synthesis by RNA polymerases on DNA templates containing several types of bifunctional adducts of platinum complexes can be prematurely terminated at the level or in the proximity of adducts (*19*, *24*, *30*−*33*). Interestingly, monofunctional DNA adducts of several platinum complexes are unable to terminate RNA synthesis (*19*, *20*, *30*). Cutting of pSP73KB DNA by *Nde*I and *Hpa*I restriction endonucleases yielded a 212-bp fragment that contained T7 RNA polymerase promoter. The experiments were carried out using this linear DNA fragment, modified by 1,2/t,c or 1,2/c,c complex at $r_b = 0.005$, for RNA synthesis by T7 RNA polymerase. RNA synthesis on the DNA template modified by these complexes yielded fragments of defined sizes, which indicates that RNA synthesis on these templates was prematurely terminated. The major stop sites produced by both trifunctional complexes 1,2/t,c and 1,2/c,c were very similar to those produced by bifunctional 1,1/t,t and 1,1/c,c, respectively (*23*, *24*), and the corresponding bands on the autoradiogram had similar intensity (data not shown). Hence, the sequence analysis has revealed that the main bands resulting from termination of RNA synthesis by 1,2/t,c or 1,2/c,c adducts preferentially appeared one or half nucleotide preceding G sites such as when RNA synthesis was analyzed on the template modified by cisplatin (*20*). Importantly, the termination sites on the DNA template preferentially produced by the adducts of dinuclear trifunctional complexes were guanine residues. These results also suggest that the adducts formed by these complexes and their relative frequency may be similar to those formed by the dinuclear bifunctional compounds 1,1/t,t and 1,1/c,c, respectively.

Unwinding Induced in Supercoiled DNA. Electrophoresis in native agarose gel was used to quantify the unwinding induced in pUC19 plasmid by the platinum complexes by monitoring the degree of supercoiling (Figure 4). Figure 4 shows electrophoresis gels in which increasing amounts of 1,2/t,c or 1,2/c,c have been bound to a mixture of nicked

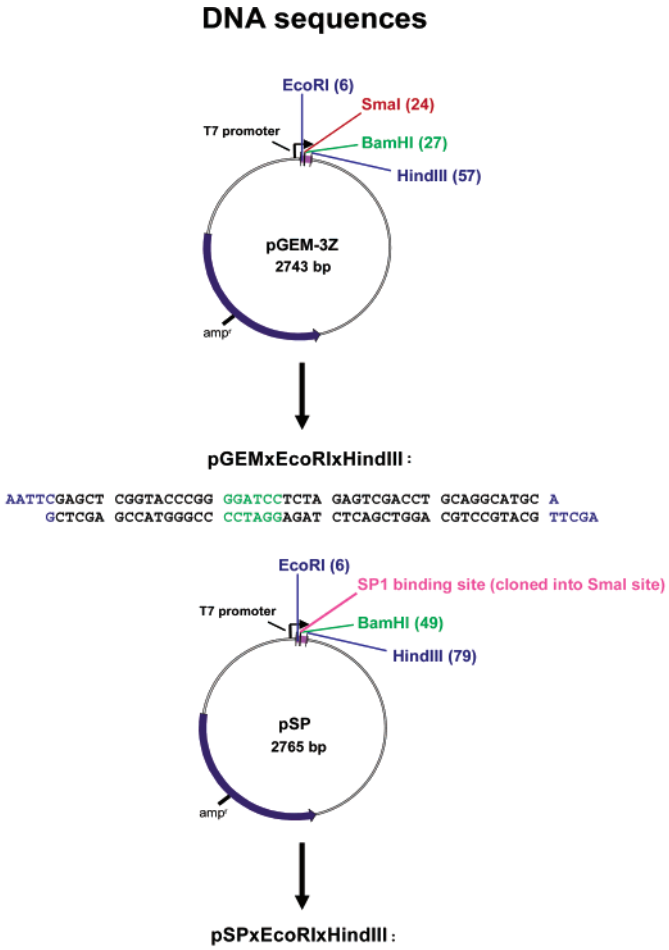


FIGURE 5: DNA sequences used for assessment of ternary DNA−protein cross-linking by trifunctional platinum compounds.

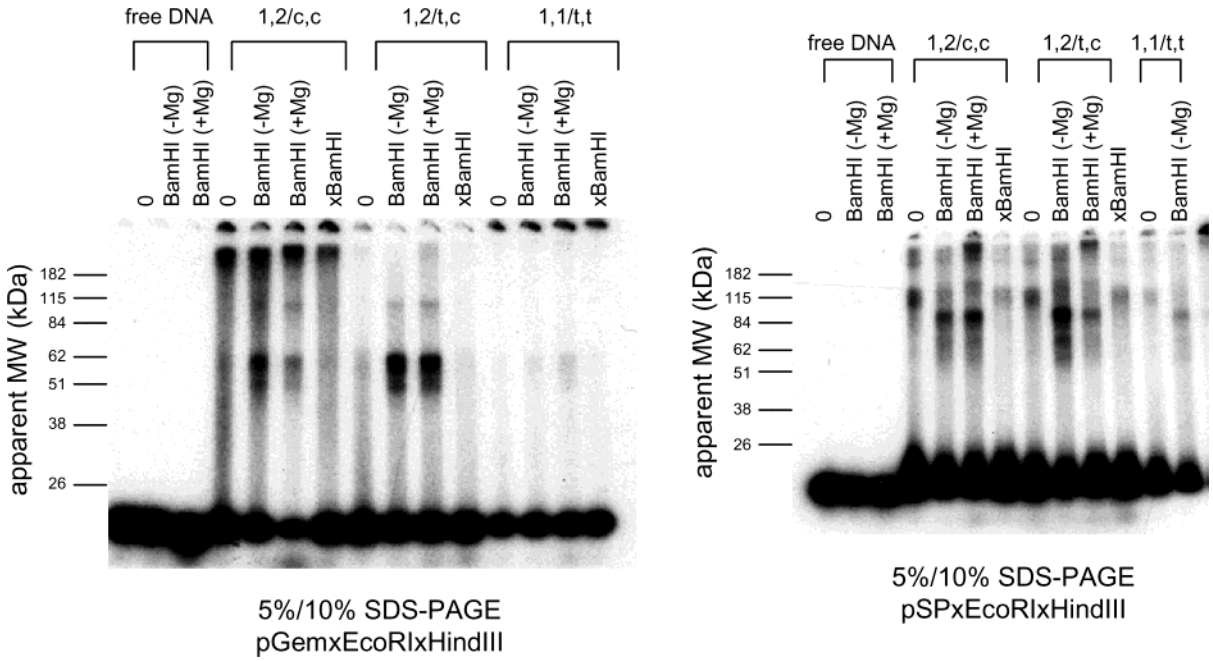


FIGURE 6: Ternary complex formation of unmodified and platinated duplexes (approximately 1 adduct/duplex) with *Bam*HI (20 μM): (−Mg) lanes = no Mg^{2+} present in the buffer; (+Mg) lanes = 10 mM $MgSO_4$ was added following preincubation of the platinated DNA with *Bam*HI; x*Bam*HI = control cleavage reactions (no preincubation with *Bam*HI). Note the limited cleavage of the platinated duplexes, presumably as a result of steric hindrance at the *Bam*HI binding site, and the very weak binding of *Bam*HI to 1,1/t,t ($n = 4$).

and supercoiled pUC19 DNA. Interestingly, the dinuclear trifunctional complexes accelerated the mobility of the relaxed form similarly as do several other bifunctional complexes, whose bifunctional binding to DNA shortens and condenses the DNA helix (*34*, *35*). The unwinding angle is given by $\Phi = 18\ \sigma/r_b(c)$ where σ is the superhelical density and $r_b(c)$ is the value of r_b at which the supercoiled and relaxed forms comigrate (*22*). Under the present experimental conditions, σ was calculated to be −0.055 on the basis of the data of cisplatin for which the $r_b(c)$ was determined in this study and $\Phi = 13°$ was assumed (*22*, *36*). Using this approach, the DNA unwinding angle of $10 \pm 1°$ was determined for both 1,2/t,c or 1,2/c,c complexes.

The values of unwinding angles are affected by the nature of the ligands in the coordination sphere of platinum and the stereochemistry at the platinum center (*22*). Platinum compounds which bind in a bifunctional manner unwind DNA by 10−13°. Examples include cisplatin, its trans isomer, and bifunctional polynuclear complexes such as 1,1/t,t, 1,1/c,c, or BBR3464 (*22*, *23*, *27*, *37*). The observation that dinuclear trifunctional complexes can be grouped with other bifunctional platinum(II) compounds is readily understood in terms of adduct structures in which the complex is preferentially coordinated to DNA in a bifunctional manner and is again consistent with the view that dinuclear trifunctional complexes form on DNA similar adducts to their bifunctional counterparts.

Interstrand Cross-Linking. The formation of site-specific 1,4-interstrand cross-links by bifunctional polynuclear platinum compounds has been confirmed by a number of techniques including NMR spectroscopy (*38*, *39*). To quantitate this feature pSP73KB plasmid (2455 bp) was linearized by *Eco*RI (*Eco*RI cuts only once within pSP73KB plasmid) and modified by the dinuclear trifunctional platinum complexes at various r_b. The samples were analyzed for the interstrand CLs by agarose gel electrophoresis under denaturing conditions (*19*). Upon electrophoresis under denaturing conditions, 3′-end labeled strands of linearized pSP73KB plasmid containing no interstrand cross-links migrate as a 2455-nucleotide single strand, whereas the interstrand cross-linked strands migrate more slowly as a higher molecular mass species. Bands corresponding to more slowly migrating interstrand-cross-linked fragments were seen for r_b values as low as 1×10^{-6} (data not shown). The intensity of the more slowly migrating band increased with the growing level of the modification. The radioactivity associated with the individual bands in each lane was measured to obtain estimates of the fraction of non-cross-linked or cross-linked DNA under each condition. The frequency of interstrand CLs was calculated using the Poisson distribution from the fraction of non-cross-linked DNA in combination with the r_b values and the fragment size. The interstrand CL frequency decreased with growing r_b. For instance, at $r_b = 5 \times 10^{-5}$, 85% and 82% of the molecules of linearized DNA were cross-linked due to the modification by 1,2/t,c or 1,2/c,c, respectively (Table 1). Thus, the DNA interstrand cross-linking efficiency of 1,2/t,c or 1,2/c,c compounds was significantly higher than that of cisplatin (6%; *19*) but similar to that of the dinuclear bifunctional complexes such as 1,1/t,t or 1,1/c,c (80−90%; *23*, *24*).

Ternary DNA−Protein Complex Formation. The trifunctional platinum compounds were then investigated for their ability to act as specific protein−DNA cross-linking agents. The bifunctional compound $[\{trans\text{-}[PtCl(NH_3)_2\}_2\mu\text{-}NH_2(CH_2)_4NH_2]^{2+}$ (1,1/t,t $n = 4$) was used as a control to compare with previous results (*6*). The 5′-end-labeled *Eco*R1−*Hin*dIII fragments from pGem and pSP plasmid DNAs, the sequences of which are given in Figure 5, were globally modified by the complexes and ternary DNA−protein cross-linking efficency was assessed by gel mobility shift assays. Platinated DNA from both trifunctional complexes efficiently bind and cross-link *Bam*H1, a sequence-

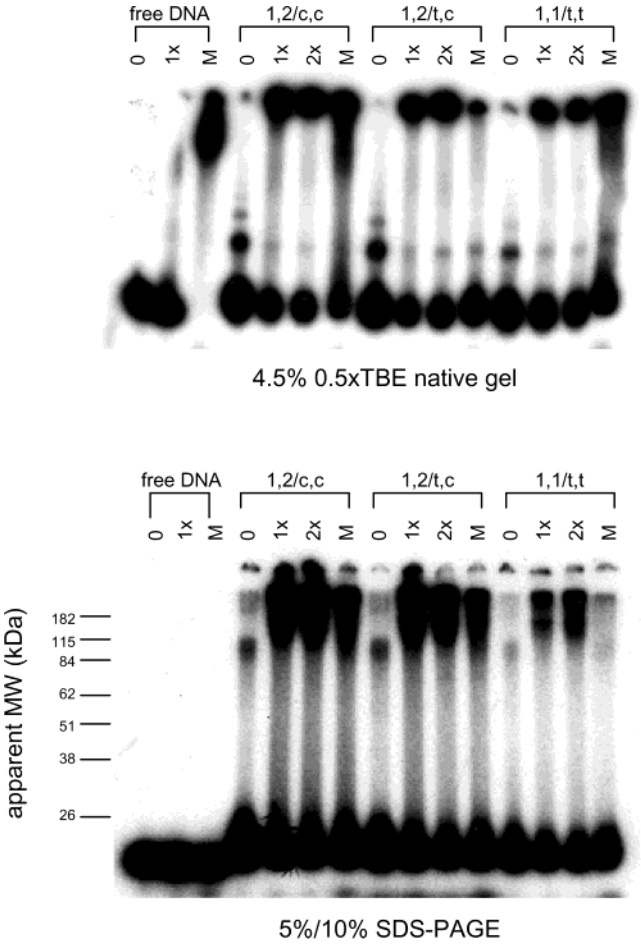


FIGURE 7: Gel shift assays of ternary complex formation of unmodified and platinated duplexes (approximately 1 adduct/duplex) with Sp1: $1x = 60$ nM Sp1 (in modified storage buffer); $2x = 120$ nM SP1 (in modified storage buffer); $M = 60$ nM Sp1 (in manufacturer's storage buffer, includes 2 mM DTT). DNA modified by the trifunctional 1,2/c,c and 1,2/t,c complexes do not inhibit Sp1 binding to its cognate site (native gel). The formation of ternary cross-linked complexes with quantitative yields is apparent under denaturing conditions (SDS−PAGE gel). DNA platinated with 1,1/t,t binds Sp1 approximately 50% as well; the presence of a small amount of cross-linked species suggests the presence of 1,1/t,t-DNA monofunctional adducts.

specific DNA-binding protein (Figure 6) as well as Sp1, a sequence-specific Zn finger protein that induces a bend in DNA upon binding (Figure 7). In the case of *Bam*H1, the observation of gel shifts in the native gels indicates that the formation of interstrand cross-links inhibits the restriction endonuclease activity of the enzyme (data not shown). The restriction site for *Bam*H1, -GGTACC-, is a possible binding site for *cis*-DDP and congeners, containing the dominant intrastrand -GG- sequence; inhibition of nuclease activity has been used as a probe of platinum binding (*40*, *41*), including [{*cis*-$Pt(NH_3)Cl_2$}$_2\mu$-$H_2N(CH_2)_4NH_2$] (*42*). Modification of DNA likewise does not inhibit Sp1 binding to its cognate site (Figure 7). Sp1 binding to the 1,1/t,t-adducted DNA is approximately 50% that of the trifunctional complexes. Under denaturing conditions, the presence of ternary covalent DNA−protein complexes is observed. For the trifunctional compounds ternary DNA−protein cross-link formation is essentially quantitative—a small amount of cross-linked species in the 1,1/t,t case suggests the presence of a small amount of monofunctional adducts. This behavior is consistent with our previous reports. Representative non-sequence-specific DNA-binding proteins, the Klenow fragment from DNA polymerase I and Klenow exonuclease minus (mutated to remove the 3′−5′ proofreading domain), both also effectively cross-link DNA modified by the 1,2 compounds, as monitored by standard gel shift assays (Figure 8). Quantitation indicated that approximately 50% of the Pt−DNA complexes are cross-linked by the 1,2/c,c and 1,2/t,c while less than 5% of the 1,1/t,t-adducted DNA forms ternary complexes, again attributed to a small amount of monofunctional adduct present.

Thus, in all cases studied it is clear that ternary DNA−protein cross-link formation is highly efficient for trifunctional complexes. Coupled with previous results using the UvrA/B repair protein complex, the range of proteins accessible to ternary DNA−protein cross-linking is large. To examine the possibility of using the trifunctional compounds as probes of protein−DNA interactions in situ, DNA and protein were incubated for 15 min after which the dinuclear compounds were added and the drug/DNA/protein mixture was incubated for 1 h as usual. The results in Figure 9 show some discrimination between proteins—especially it appears that both 1,2/t,c and 1,2/c,c are capable of specifically cross-linking the *Bam*H1 protein with the formation of covalent ternary adducts (see circled region in Figure 9). The highlighted spot is similar to that formed by incubating the preformed Pt−DNA complex with protein (Figure 6). Allowing for only one dinuclear platinum adduct per plasmid (which is controlled by selection of r_b), predicted molecular weights of the covalently cross-linked ternary complex (i.e., 1:1:1) for *Bam*H1 are 58.9 and 73.2 kDa for the pGEM×*Eco*RI×*Hin*dIII and pSP×*Eco*RI×*Hin*dIII plasmids, respectively. Apparent molecular weights are in good agreement with these predictions for both the case of incubation of protein with platinated DNA (Figure 6) and the in situ experiment of Figure 9.

DISCUSSION

Model studies using 5′-GMP indicated that, for both 1,2/t,c and 1,2/c,c, the monofunctional [$Pt(amine)_3Cl$] coordination sphere reacted faster than the bifunctional *cis*-[$Pt(amine)_2Cl_2$] end and is the first site of substitution. Extension to polynucleotide DNA would automatically set up an interstrand cross-link in a second step. The physical and biochemical studies performed here are entirely consistent with long-range(Pt,Pt) interstrand cross-links as the predominant lesion in trifunctional compounds (Table 1). It is not possible to unambiguously rule out some formation of *cis*-DDP-like intrastrand adducts—some -GG- sites stop sites are observed with the transcription mapping assay (Figure 5), which may be attributed to intrastrand adduct formation. However, previous studies using a novel interstrand cross-link formation assay showed that -GG- stop sites in G-rich regions may in fact be misinterpreted as intrastrand rather than interstrand cross-links (*2*). Furthermore, using the bifunctional [{$PtCl(NH_3)_2$}μ-$H_2N(CH_2)_6NH_2$]$^{2+}$, competitive binding studies using HSQC {^{1}H,^{15}N} NMR spectroscopy showed preferential formation of interstrand rather than intrastrand adducts within the 14-mer duplex (*43*). Thus, intrastrand adducts of trifunctional complexes, if present, are likely to represent only a very small proportion of the total and contribute little to the conformational distortions ob-

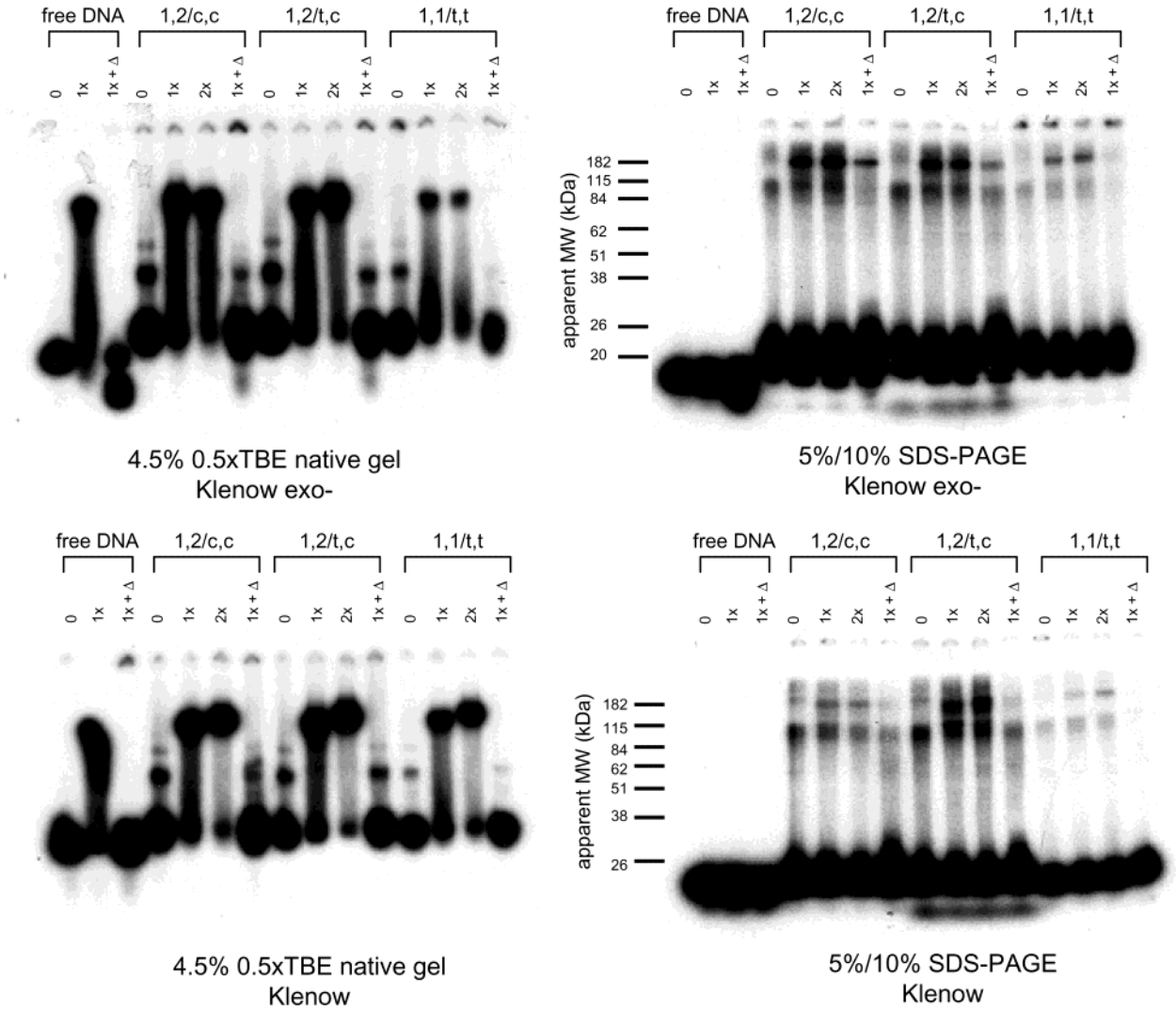


FIGURE 8: Ternary complex formation of unmodified and platinated duplexes (approximately 1 adduct/duplex) with Klenow or Klenow exo$^-$: $1x = 5\ \mu$M protein; $2x = 10\ \mu$M protein; $1x + \Delta =$ samples were treated with loading buffer containing DTT and heated at 90 °C for 5 min prior to loading. Both Klenow and Klenow exo$^-$ bind all three platinated duplexes; approximately 50% of the complexes are cross-linked by 1,2/c,c and 1,2/t,c, while only 10% are cross-linked by 1,1/t,t.

served. Further studies using the reported [*trans*-{PtCl-$(NH_3)_2$}μ-$H_2(CH_2)_6NH_2$-{*cis*-Pt(NH_3)(malonate)}] (*13*) will eventually resolve this issue because of the large kinetic difference between Pt−Cl and Pt−dicarboxylate displacement and will produce even more selective interstrand cross-linking agents, capable of further selective reaction with biomolecules.

It is of interest, therefore, to consider DNA−protein binding in the context of specific long-range cross-links. Complementary physical chemical, spectroscopic, and molecular biological evidence confirms the conformational flexibility of long-range (Pt,Pt) interstrand cross-links. The presence of only one guanine base on each Pt center removes the steric constraints present when two guanine nucleobases must bind to the cis positions of a mononuclear Pt center, thus negating one driving force for helix bending. Rather than a rigid, directed bending, interstrand cross-linking "locks in" unusual DNA conformations (*44*, *45*) and may produce cooperative effects extending the effect of the lesion well past the physical binding site (*38*). It is probable that the interstrand cross-links of trifunctional compounds are very similar in structure to those formed by 1,1/t,t and 1,1/c,c. Molecular modeling and preliminary studies on site-specific oligonucleotide interstrand cross-links confirm this conclusion. The conformational flexibility ensures that in general the bifunctional 1,1/t,t adducts are poor substrates for recognition by proteins that bind to rigidly bent DNA, such as those containing the HMG domain (*9*, *11*). In agreement, HMG domain proteins bind only weakly to DNA modified by the trinuclear complexes (data not shown). The observation of ternary DNA−protein complex formation with Sp1 is therefore noteworthy as the protein induces bending in DNA upon binding (*46*). A multitude of proteins have been shown to recognize DNA modified by the anticancer drug *cis*-DDP (*47*−*50*). Many of these proteins have been shown to contain the HMG domain motif, but to our knowledge, no assay of Sp1 binding to plasmid DNA adducted by *cis*-DDP has been reported. However, in studies on the effect of chromatin structure on cisplatin damage in intact human cells, enhanced damage at a -CACC- site (where Sp1 proteins are believed to bind) was found (*51*). It was suggested as one explanation of this phenomenon that "prebending" may lead to enhanced DNA binding at the site of bending. The demonstration of in situ ternary DNA−protein cross-linking in the present experiments suggests that this postulate may be feasible. Further circumstantial evidence for the impor-

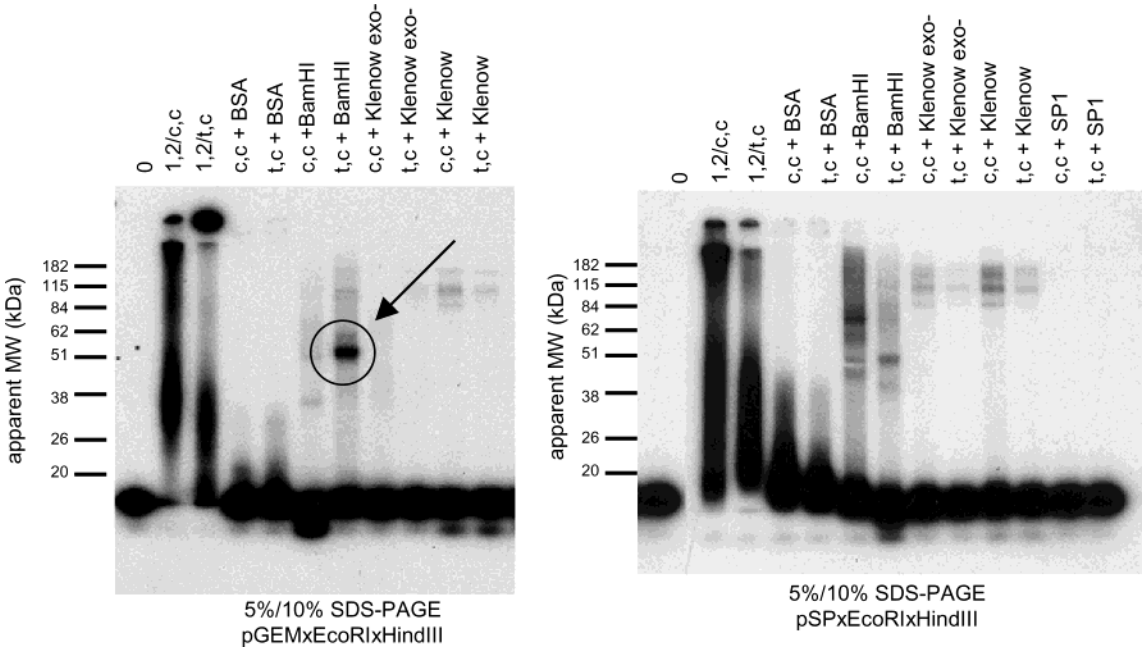


FIGURE 9: In situ ternary DNA−protein cross-link formation induced by trifunctional dinuclear platinum complexes (c,c is 1,2/c,c and t,c is 1,2/t,c in these legends). Uncomplexed duplexes were treated with indicated proteins in 10 mM Tris-HCl, pH 8, 0.1 mM EDTA, 0.8% glycerol, and 0.1 μg of BSA (supplemented with 2 mM $MgSO_4$ for Klenow exo$^-$ and SP1 reactions) at 25 °C for 15 min. Drug (10 μM final concentration) was added, and the reaction was incubated at 25 °C for 1 h. Note that both 1,2/c,c and 1,2/t,c are able to specifically cross-link the protein to the DNA.

tance of Sp1 interactions with cisplatin-damaged DNA comes from observations that, in vitro, *cis*-DDP attacks the GC-rich control elements within the regulatory region of the DNA tumor virus SV40 (*52*, *53*). Previous demonstrations of recognition and excision of Pt-adducted DNA by the bacterial UvrABC repair system are relevant because zinc fingers are also present in the active sites of these proteins (*6*, *54*). The structural details of such recognition are worth exploring in comparison to those of the HMG domain family (*55*). For the case of dinuclear platinum complexes, the differential recognition between classes of proteins responsible for DNA bending may help to distinguish on a molecular basis the intracellular pathways to repair and apoptosis in comparison to *cis*-DDP.

Ternary DNA−protein complexes are also formed by mononuclear *cis*-DDP. Control of conditions allows observation of cross-linking of HMG group proteins 1 and 2 to DNA in micrococcal nuclease accessible regions of chromatin (*56*). DNA modified by cisplatin may also undergo photoinduced cross-linking to HMG1 proteins upon irradiation (*57*). Incorporation of steric hindrance into an ethylenediamine chelate may also allow for ternary DNA−protein cross-linking due to retardation of the second DNA binding step and favorable competitive reaction with protein (*58*). In all these cases protein fixation comes from monofunctional platinated DNA, yet monofunctional binding involves no major conformational distortion (*59*). Thus, it would be expected that little discrimination would be seen in ternary DNA−protein complex formation.

The cytotoxicity profile of the trifunctional compounds in a panel of human ovarian cancer cell lines is also somewhat different from that of the bifunctional analogues (*12*). The formation of metal-mediated DNA−protein ternary complexes raises the possibility of "suicide" lesions, which may irreversibly sequester a repair protein or transcription factor (*60*). In situ, cross-linking of nuclear matrix-bound transcription factors has been observed in nuclear DNA of human breast cancer cells (*61*). It is likely that structures and consequences of DNA−protein cross-links will differ between that of mononuclear (*62*) and dinuclear compounds, suggesting further rational pathways to separate the biological consequences of the two drug classes.

REFERENCES

1. Farrell, N. (1995) DNA binding and chemistry of dinuclear platinum complexes, *Comments Inorg. Chem. 16*, 373−389.
2. Zou, Y., Vanhouten, B., and Farrell, N. (1994) Sequence specificity of DNA−DNA interstrand cross-link formation by cisplatin and dinuclear platinum complexes, *Biochemistry 33*, 5404−5410.
3. Farrell, N. (1996) DNA binding of dinuclear platinum complexes, in *Advances in DNA Sequence Specific Agents* (Hurley, L. H., Chaires, J. B., Eds.) Vol. 2, pp 187−216, JAI Press Inc., London.
4. Farrell, N. (1998) DNA binding of non-classical platinum antitumor complexes, in *Advances in DNA Sequence Specific Agents* (Palumbo, M., Ed.) Vol. 3, pp 179−199, JAI Press Inc., London.
5. Farrell, N., Qu, Y., and Roberts, J. D. (1999) Multifunctional DNA-binding metal complexes, in *Topics in Biological Inorganic Chemistry* (Clarke, M. J., and Sadler, P. J., Eds.) pp 99−115, Springer, New York.
6. Van Houten, B., Illenye, S., Qu, Y., and Farrell, N. (1993) Homodinuclear (Pt,Pt) and heterodinuclear (Ru,Pt) metal complexes as DNA−protein cross-linking agents: Potential suicide DNA lesions, *Biochemistry 3*, 11794.
7. Qu, Y., and Farrell, N. (1995) Synthesis and chemical properties of a heterodinuclear (Pt,Ru) DNA−DNA and DNA−protein cross-linking agent, *Inorg. Chem. 34*, 3573.
8. Qu, Y., Bloemink, M. J., Mellish, K. J., Rauter, H., Smeds, K. A., and Farrell, N. (1997) Factors affecting formation and structure of DNA intrastrand cross-links by dinuclear platinum complexes, in *Cytotoxic, Mutagenic and Carcinogenic Potential of Heavy Metals Related to Human Environment* (Hadjiliadis, N., Ed.) NATO ASI Series 2, Vol. 26, p 435−444, Kluwer Academic, Dordrecht.
9. Kasparkova, J., Farrell, N., and Brabec, V. (2000) Sequence specificity, conformation and recognition by HMG1 protein of major DNA interstrand cross-links of antitumor dinuclear platinum complexes, *J. Biol. Chem. 275*, 15789−15798.

10. Roberts, J. D., Peroutka, J. and Farrell, N. (1999) Cellular pharmacology of polynuclear platinum anti-cancer agents, *J. Inorg. Biochem. 77*, 51−57.
11. Kasparkova, J., Zehnulova, J. Farrell, N., and Brabec, V. (2002) DNA interstrand cross-links of novel antitumor trinuclear platinum complex BBR3464. conformation, recognition by HMG-domain proteins and nucleotide excision repair, *J. Biol. Chem*. 48076−48086.
12. Qu, Y., Rauter, H., Soares Fontes, A. P., Bandarage, R., Kelland, L. R., and Farrell, N. (2000) Synthesis, characterization and cytotoxicity of trifunctional dinuclear platinum complexes. A comparison of effects of geometry and polyfunctionality on biological activity, *J. Med. Chem. 43*, 3189−3192.
13. Qu, Y., Fitzgerald, J. A., Rauter, H., and Farrell, N. (2001) Approaches to selective DNA binding in polyfunctional dinuclear platinum chemistry. The synthesis of a trifunctional compound and its interaction with the mononucleotide 5′-guanosine monophosphate, *Inorg. Chem. 40*, 6324−6327.
14. Brabec, V., and Palecek, E. (1970) The influence of salts and pH on polarographic currents produced by denatured DNA, *Biophysik 6*, 290−300.
15. Brabec, V., and Palecek, E. (1976) Interaction of nucleic acids with electrically charged surfaces. II. Conformational changes in double-helical polynucleotides, *Biophys. Chem. 4*, 76−92.
16. Kim, S. D., Vrana, O., Kleinwächter, V., Niki, K., and Brabec, V. (1990) Polarographic determination of subnanogram quantities of free platinum in reaction mixture with DNA. *Anal. Lett. 23*, 1505−1518.
17. Butour, J. L., and Macquet, J. P. (1977) Differentiation of DNA−platinum complexes by fluorescence. The use of an intercalating dye as a probe, *Eur. J. Biochem. 78*, 455−463.
18. Butour, J. L., Wimmer, S., Wimmer, F., and Castan, P. (1997) Palladium(II) compounds with potential antitumour properties and their platinum analogues: A comparative study of the reaction of some orotic acid derivatives with DNA in vitro, *Chem.−Biol. Interact. 104*, 165−178.
19. Brabec, V., and Leng, M. (1993) DNA interstrand cross-links of *trans*-diamminedichloroplatinum(II) are preferentially formed between guanine and complementary cytosine residues, *Proc. Natl. Acad. Sci. U.S.A. 90*, 5345−5349.
20. Lemaire, M. A., Schwartz, A., Rahmouni, A. R., and Leng, M. (1991) Interstrand cross-links are preferentially formed at the d(GC) sites in the reaction between *cis*-diamminedichloroplatinum-(II) and DNA, *Proc. Natl. Acad. Sci. U.S.A. 88*, 1982−1985.
21. Farrell, N., Qu, Y., Feng, L., and Van Houten, B. (1990) Comparison of chemical reactivity, cytotoxicity, interstrand cross-linking and DNA sequence specificity of bis(platinum) complexes containing monodentate or bidentate coordination spheres with their monomeric analogues, *Biochemistry 29*, 9522−9531.
22. Keck, M. V., and Lippard, S. J. (1992). Unwinding of supercoiled DNA by platinum ethidium and related complexes, *J. Am. Chem. Soc. 114*, 3386−3390.
23. Kasparkova, J., Novakova, O., Vrana, O., Farrell, N., and Brabec, V. (1999) Effect of geometric isomerism in dinuclear platinum antitumor complexes on DNA interstrand cross-linking, *Biochemistry 38*, 10997−11005.
24. Zaludova, R., Zakovska, A., Kasparkova, J., Balcarova, Z., Kleinwächter, V., Vrana, O., Farrell, N., and Brabec, V. (1997) DNA interactions of bifunctional dinuclear platinum(II) antitumor agents, *Eur. J. Biochem. 246*, 508−517.
25. Zaludova, R., Kleinwächter, V., and Brabec, V. (1996) The effect of ionic strength on melting of DNA modified by platinum(II) complexes, *Biophys. Chem. 60*, 135−142.
26. Brabec, V., Kleinwächter, V., Butour, J. L., and Johnson, N. P. (1990) Biophysical studies of the modification of DNA by antitumour platinum coordination complexes, *Biophys. Chem. 35*, 129−141.
27. Brabec, V., Kasparkova, J., Vrana, O., Novakova, O., Cox, J. W., Qu, Y., and Farrell, N. (1999) DNA modifications by a novel bifunctional trinuclear platinum Phase I anticancer agent, *Biochemistry 38*, 6781−6790.
28. Brabec, V., Vrána, O., Nováková, O., Kleinwächter, V., Intini, F. P., Coluccia, M., and Natile, G. (1996) DNA adducts of antitumor *trans*-[$PtCl_2$(E-imino ether)$_2$], *Nucleic Acids Res. 24*, 336−341.
29. Zakovska, A., Novakova, O., Balcarova, Z., Bierbach, U., Farrell, N., and Brabec, V. (1998) DNA interactions of antitumor *trans*-[$PtCl_2(NH_3)$(quinoline)], *Eur. J. Biochem. 254*, 547−557.
30. Brabec, V., Boudny, V., and Balcarova, Z. (1994) Monofunctional adducts of platinum(II) produce in DNA a sequence-dependent local denaturation, *Biochemistry 32*, 1316−1322.
31. Corda, Y., Anin, M. F., Leng, M., and Job, D. (1992) RNA polymerases react differently at d(ApG) and d(GpG) adducts in DNA modified by *cis*-diamminedichloroplatinum(II), *Biochemistry 31*, 1904−1908.
32. Corda, Y., Job, C., Anin, M. F., Leng, M., and Job, D. (1991) Transcription by eucaryotic and procaryotic RNA polymerases of DNA modified at a d(GG) or a d(AG) site by the antitumor drug *cis*-diamminedichloroplatinum(II), *Biochemistry 30*, 222−230.
33. Novakova, O., Vrana, O., Kiseleva, V. I., and Brabec, V. (1995) DNA interactions of antitumor platinum(IV) complexes, *Eur. J. Biochem. 228*, 616−624.
34. Cohen, G. L., Bauer, W. R., and Lippard, S. J. (1979) Binding of cis and trans dichlorodiammineplatinum(II) to DNA: Evidence for unwinding and shortening of the double helix, *Science 203*, 1014−1016.
35. Bellon, S. F., Coleman, J. H., and Lippard, S. J. (1991) DNA unwinding produced by site-specific intrastrand cross-links of the antitumor drug *cis*-diamminedichloroplatinum(II), *Biochemistry 30*, 8026−8035.
36. Scovell, W. M., and Collart, F. (1985) Unwinding of supercoiled DNA by *cis*- and *trans*-diamminedichloro-platinum(II): Influence of the torsional strain on DNA unwinding, *Nucleic Acids Res. 13*, 2881−2895.
37. Farrell, N., Appleton, T. G., Qu, Y., Roberts, J. D., Fontes, A. P. S., Skov, K. A., Wu, P., and Zou, Y. (1995) Effects of geometric isomerism and ligand substitution in bifunctional dinuclear platinum complexes on binding properties and conformational changes in DNA, *Biochemistry 34*, 15480−15486.
38. Qu, Y., Scarsdale, N. J., Tran, M.-C., and Farrell, N. (2003) Cooperative effects in long-range 1,4 DNA−DNA interstrand cross-links formed by polynuclear platinum complexes. An unexpected *syn*-orientation of adenine bases outside the binding sites, *J. Biol. Inorg. Chem. 8*, 19−28.
39. Cox, J. W., Berners-Price, S. J., Davies, M. S., Qu, Y. and Farrell, N. (2001) Kinetic analysis of the stepwise formation of a long-range DNA interstrand cross-link by a dinuclear platinum antitumor complex: Evidence for aquated intermediates and formation of both kinetically and thermodynamically controlled conformers, *J. Am. Chem. Soc. 123*, 1316−1326.
40. Scovell, W. M., Kroos, L. and Capponi, V. (1983) In *Platinum, Gold and Other Metal Chemotherapeutic Agents*; Lippard, S. J., Ed.; ACS Symposium Series 209; p 101, American Chemical Society, Washington, DC.
41. Skov, K. A., Adomat, H., Konway, D. C., and Farrell, N. (1987) Assessment of DNA binding of platinum-radiosensitizer complexes by inhibition of restriction enzymes, *Chem.−Biol. Interact. 62*, 117−129.
42. deAlmeida, S. G., Skov, K. A., and Farrell, N. P. (1988) Bisplatinum complexes containing two *cis*-Pt(amine)$_2$ units. Synthesis and initial DNA-binding studies, *J. Am. Chem. Soc. 110*, 5018−5019.
43. Berners-Price, S. J., Davies, M. S. Cox, J. W., Thomas, D. S. and Farrell, N. (2003) Competitive reactions of interstrand and intrastand DNA−Pt adducts. A dinuclear platinum complex preferentially forms a 1,4 interstrand cross-link rather than a 1,2 intrastrand cross-link on binding to a GG 14-mer duplex, *Eur. J. Chem. 9*, 713−725.
44. McGregor, T. D., Bousfield, W., Qu, Y., and Farrell, N. (2002) Circular dichroism study of the irreversibility of conformational changes induced by polyamine-linked dinuclear platinum compounds, *J. Inorg. Biochem. 91*, 212−219.
45. McGregor, T. D., Balcarova, Z., Qu, Y., Tran, M.-C., Zaludova, R., Brabec, V., and Farrell, N. (1999) Sequence dependent conformational changes in DNA induced by the trinuclear platinum complex BBR3464, *J. Inorg. Biochem. 77*, 43−46.
46. Suske, G. (1999) The Sp-family of transcription factors, *Gene 238*, 291−300.
47. Kartalou, M., and Essigmann, J. M. (2001) Recognition of cisplatin adducts by cellular proteins, *Mutat. Res. 478*, 1−21.
48. Jamieson E., and Lippard, S. J. (1999) Structure, recognition, and processing of cisplatin−DNA adducts, *Chem. Rev. 99*, 2467−2498.
49. Zlatanova, J., Yaneva, J., and Leuba, S. H. (1998) Proteins that specifically recognize cisplatin-damaged DNA: A clue to anticancer activity of cisplatin, *FASEB J. 12*, 791−799.

50. Chu, G. (1994) Cellular responses to cisplatin, *J. Biol. Chem. 269*, 787−790.
51. Davies, N. P., Hardman, L. C., and Murray, V. (2000) The effect of chromatin structure on cisplatin damage in intact human cells, *Nucleic Acids Res. 28*, 2954−2958.
52. Buchanan, R. L., and Gralla, J. D. (1990) Cisplatin resistance and mechanism in a viral test system: SV40 isolates that resist inhibition by the antitumor drug have lost regulatory DNA, *Biochemistry 29*, 3436−3442.
53. Gralla, J. D., Sasse-Dwight, S., and Poljak, L. G. (1987) Formation of blocking lesions at identical DNA sequences by the nitrosourea and platinum classes of anticancer drugs. *Cancer Res. 47*, 5092−5096.
54. Sancar A. (1996) DNA excision repair, *Annu. Rev. Biochem. 65*, 43−81.
55. Ohndorf, U.-M., Rould, M. A., He, Q., Pabo, C. O., and Lippard, S. J. (1999) Basis for recognition of cisplatin-modified DNA by high-mobility-group proteins. *Nature 399*, 708−712.
56. Scovell, W. M., Muirhead, N., and Kroos, L. R. (1987) Cis-diamminedichloroplatinum(II) selectively cross-links high mobility group proteins 1 and 2 to DNA in micrococcal nuclease accessible regiosn of chromatin, *Biochem. Biophys. Res. Commun. 142*, 826.
57. Kane, S. A., and Lippard, S. J. (1996) Photoreactivity of platinum-(II) in cisplatin-modified DNA affords specific cross-links to HMG domain proteins, *Biochemistry 35*, 2180−2188.
58. Lambert, B., Jestin, J.-L., Brehin, P., Oleyowski, C., Yeung, A., Mailliet, P., Pretot, C., le Pecq, J.-B., Jacquemin-Sablon, A., and Chottard, J.-C. (1995) Binding of the *Escherichia coli* UvrAB proteins to the DNA mono- and diadducts of *cis*-[*N*-2-amino-*N*-2-methylamino-2,2,1-bicycloheptane]dichloroplatinum(II) and cis-platin. Analysis of the factors controlling recognition and proof of monoadduct-mediated UvrB-DNA-*cross*-linking, *J. Biol. Chem. 270*, 21251.
59. Brabec, V., Reedijk, J., and Leng, M. (1992) Sequence-dependent distortions induced in DNA by monofunctional platinum(II) binding, *Biochemistry 31*, 12397−12402.
60. Rink, S. M., Yarema, K. J., Solomon, M. S., Paige, L. A., Tadayoni-Rebek, B. M., Essigmann, J. M., and Croy, R. G. (1996) Synthesis and biological activity of DNA damaging agents that form decoy binding sites for the estrogen receptor, *Proc. Natl. Acad. Sci. U.S.A. 93*, 15063−15068.
61. Samuel, S. K., Spencer, V. A., Bajno, L., Sun, J.-M., Holth, L. T., Oesterreich, S., and Davie, J. R. (1998) In situ cross-linking by cisplatin of nuclear matrix-bound transcription factors to nuclear DNA of human breast cancer cells, *Cancer Res. 58*, 3004−3008.
62. Wozniak, K., and Walter, Z. (2000) Induction of DNA−protein cross-links by platinum compounds, *Z. Naturforsch. 55*, 731−736.
63. Bancroft, D. P., Lepre, C. A., and Lippard, S. J. (1990) Pt-195 NMR kinetic and mechanistic studies of *cis*-diamminedichloroplatinum and *trans*-diamminedichloroplatinum(II) binding to DNA, *J. Am. Chem. Soc. 112*, 6860−6871.
64. Johnson, N. P., Macquet, J.-P., Wiebers, J. L., and Monsarrat, B. (1982) Structures of adducts formed between [Pt(dien)Cl]Cl and DNA in vitro, *Nucleic Acids Res. 10*, 5255−5271.
65. Eastman, A. (1987) The formation, isolation and characterization of DNA adducts produced by anticancer platinum complexes, *Pharmacol. Ther. 34*, 155−166.

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