Unwinding of Supercoiled DNA by Platinum–Ethidium and Related Complexes

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Abstract: A gel electrophoretic mobility shift assay has been used to determine the unwinding of closed circular, supercoiled pUC 19 plasmid DNA induced by a variety of platinum complexes that differ in their coordination mode to the double helix. Included are the anticancer drug cisplatin and several of its analogues, compounds in which the organic intercalator ethidium is coordinated to platinum through an exocyclic amino group, and molecules in which two platinum centers are tethered by a polymethylene chain. The unwinding angles (ϕ) for the 12 compounds investigated range from 6° to 19°, with ϕ increasing in the order monofunctional binding < bifunctional binding < monofunctional plus intercalative binding < bifunctional plus intercalative binding. Unwinding titration experiments thus provide a useful screen of the binding mode of divalent platinum complexes that modify DNA. In the case of the platinum–ethidium complexes, the ϕ values provide experimental evidence for both covalent and intercalative binding of this interesting class of compounds. Information of this kind might facilitate the design of compounds, including antitumor drug candidates, that unwind DNA to any desired extent.

Introduction

Recently there has been much interest in the study of molecules that combine two or more functionalities that modify DNA. The use of metals in such compounds is well documented. Examples include methidiumpropyl-EDTA-Fe(II) (MPE), a DNA affinity cleaving agent,[1] chiral metal complexes used as probes of DNA shape,[2] and the natural product bleomycin.[3] The antitumor drug cis-diamminedichloroplatinum (cis-DDP or cisplatin) binds covalently to DNA with concomitant bending and local unwinding of the double helix.[4,5] The sequence specificity of its binding is altered in the presence of ethidium, however,[6,7] and DNA can serve as a template to promote the reaction between cis-DDP and Etd+.[8-10] The nature of the platinum–ethidium–DNA ternary

cis-DDP   Ethidium, Etd+
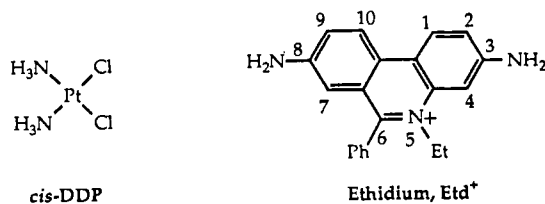
complex has been precisely defined through the synthesis of two compounds having ethidium coordinated by either of the two different exocyclic amino groups.[11] Binding of these two linkage isomers to DNA resulted in complexes having optical absorption spectra nearly identical to that of the ternary complex formed by cis-DDP and ethidium on DNA.

Subsequently, effort has been directed toward obtaining more detailed structural information on the ternary complex and, more specifically, on the distortion of the DNA induced by these platinum compounds. Such modification of DNA tertiary structure by drug binding is an important aspect of its molecular

recognition by DNA processing proteins in the cell.[12,13] As an initial probe, we have used agarose gel electrophoresis to study the unwinding of closed circular supercoiled plasmid DNA induced by these and related complexes. It has been shown previously[5,14,15] that cis-DDP and ethidium bromide[16] unwind supercoiled DNA by 13° and 26°, respectively, corresponding to covalent crosslinking and intercalation into the double helix.[17] Subsequently we have employed this methodology to measure the unwinding angles of a series of platinum complexes bound to the exocyclic amino groups of ethidium (Figure 1). During the course of this investigation, we discovered a very interesting correlation between the unwinding angles and the mode of interaction of the complexes with DNA. These results suggested that the method might be useful for evaluating the binding mode of other, potentially multifunctional, platinum complexes such as the bis(platinum) complexes containing two mono- or bifunctional moieties linked by a polymethylene tether.[18-20] We therefore expanded our study to include these compounds, as well as other cisplatin analogues, and describe the results in the present report.

Experimental Section

The synthesis and characterization of the platinum–ethidium complexes are reported elsewhere.[11,21] cis-DDP[22] and [Pt(dien)Cl]Cl[23] (dien = diethylenetriamine) were prepared by published procedures. *cis*-[Pt-(NH₃)₂(4-Br-py)Cl]Cl was provided by L. S. Hollis and used without further purification. The complexes [{Pt(mal)(NH₃)}₂H₂N(CH₂)₄NH₂] (mal = malonate) and [{trans-PtCl(NH₃)₂}₂H₂N(CH₂)₄NH₂]Cl₂ were supplied by N. P. Farrell[24] and are referred to in this paper as the bifunctional and monofunctional bis(platinum) complexes, respectively. The compound [Pt(NH₃)₃Cl]Cl was synthesized by first allowing trans-DDP (1.14 g, 3.8 mmol) to react overnight with AgNO₃ (0.64 g,

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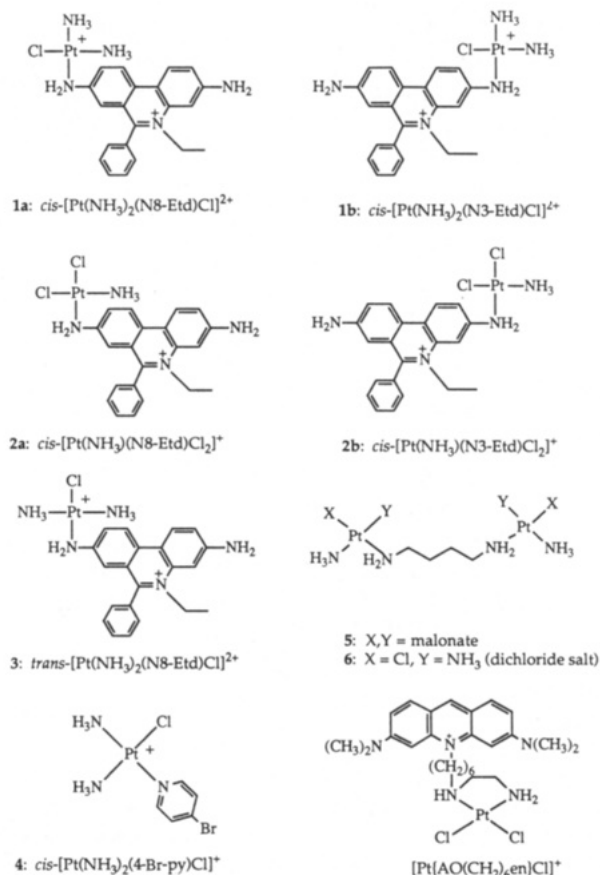(24) Qu, Y.; Farrell, N. *J. Am. Chem. Soc.* **1991**, *113*, 4851.

1a: *cis*-[Pt(NH$_3$)$_2$(N8-Etd)Cl]$^{2+}$

1b: *cis*-[Pt(NH$_3$)$_2$(N3-Etd)Cl]$^{2+}$

2a: *cis*-[Pt(NH$_3$)(N8-Etd)Cl$_2$]$^{+}$

2b: *cis*-[Pt(NH$_3$)(N3-Etd)Cl$_2$]$^{+}$

3: *trans*-[Pt(NH$_3$)$_2$(N8-Etd)Cl]$^{2+}$

5: X,Y = malonate
6: X = Cl, Y = NH$_3$ (dichloride salt)

4: *cis*-[Pt(NH$_3$)$_2$(4-Br-py)Cl]$^{+}$

[Pt{AO(CH$_2$)$_6$en}Cl]$^{+}$

Figure 1. Structures and abbreviations for platinum complexes used in this study.

3.8 mmol) in DMF (100 mL). The resulting AgCl precipitate was filtered and 2 mL of 15 N aqueous NH$_4$OH was added to the filtrate. After 1 h, the solution was evaporated to dryness in vacuo. The resulting yellow solid was taken up in 60 mL of 0.1 N HCl, concentrated by gentle heating, and precipitated by adding two volume equivalents of ethanol and cooling to −20 °C. Recrystallization from 0.1 M HCl/ethanol yielded 564 mg (47%) of [Pt(NH$_3$)$_3$Cl]Cl. ^{195}Pt NMR, −2371.5 ppm. Anal. Calcd for PtN$_3$H$_9$Cl$_2$: Pt, 61.5. Found: Pt, 62.0. Drawings and nomenclature for all platinum compounds investigated here are presented in Figure 1.

Buffers used and their abbreviations are TE, 10 mM Tris, 1 mM EDTA, pH 7.4, and TAE, 40 mM Tris acetate, 1 mM EDTA, pH 7.4. pUC 19 plasmid DNA was isolated from *E. coli* strain XL1-blue with the use of Qiagen DNA purification kits, followed by separation of the supercoiled form by CsCl density gradient centrifugation.

Platination reactions with the ethidium complexes were carried out on 5 μg of DNA in TE buffer for either 4 h at room temperature or overnight at 4 °C in the dark. Formal drug-to-nucleotide ratios (r_f) ranged from 0.01 to 0.52. Buffered solutions of the platinum complexes were freshly prepared immediately before each experiment, and their concentrations were determined by optical absorption spectroscopy with use of the following extinction coefficients: *cis*-[Pt(NH$_3$)$_2$(N8-Etd)Cl]-(OAc)$_2$, ϵ_{450} = 11 470 M^{-1} cm^{-1}; *cis*-[Pt(NH$_3$)$_2$(N3-Etd)Cl](OAc)$_2$, ϵ_{440} = 8860 M^{-1} cm^{-1}; *cis*-[Pt(NH$_3$)(N8-Etd)Cl$_2$](OAc), ϵ_{451} = 5480 M^{-1} cm^{-1}; *cis*-[Pt(NH$_3$)(N3-Etd)Cl$_2$](OAc), ϵ_{441} = 4500 M^{-1} cm^{-1}; *trans*-[Pt(NH$_3$)$_2$(N8-Etd)Cl](OAc)$_2$, ϵ_{456} = 6765 M^{-1} cm^{-1}. Platinations with *cis*-DDP, *trans*-DDP, [Pt(NH$_3$)$_3$Cl]Cl, [Pt(dien)Cl]Cl, [Pt(NH$_3$)$_2$(4-Br-py)Cl]Cl, and the monofunctional bis(platinum) complex were carried out on 5 μg of DNA for 24 h at 37 °C. Platination with the bifunctional bis(platinum) complex was carried out at 37 °C for 5 days.

After platination, all samples were dialyzed through a BRL 1202 MD dialysis membrane against a minimum quantity of 6 L of TE buffer at 4 °C in the dark in a BRL 1200 MD microdialysis apparatus. Final bound platinum concentrations were determined by flameless atomic absorption spectroscopy, and DNA concentrations were determined spectrophotometrically with the use of an extinction coefficient ϵ_{260} = 6600 M^{-1} cm^{-1}. Since both the platinum complexes and ethidium absorb at this wavelength, control experiments were carried out to ensure that this method of DNA quantitation was valid. *cis*-DDP was allowed to react with 1.95 equiv of AgNO$_3$ overnight to generate *cis*-[Pt(NH$_3$)$_2$-(OH$_2$)$_2$]$^{2+}$. This complex was then added to calf thymus DNA in TE
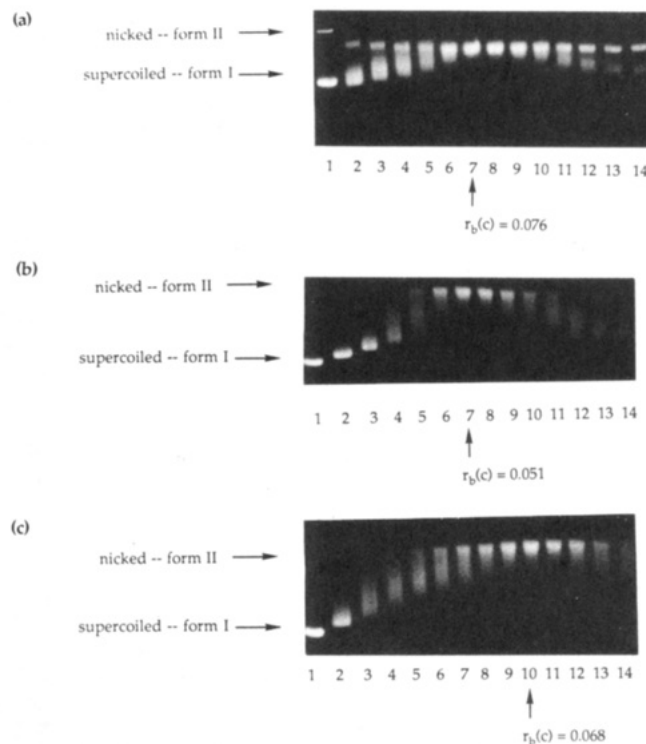
Figure 2. Titration of pUC 19 DNA: (a) *cis*-DDP, r_b levels for lanes 1–14, (1) 0.0, (2) 0.019, (3) 0.032, (4) 0.041, (5) 0.046, (6) 0.061, (7) 0.074, (8) 0.078, (9) 0.087, (10) 0.093, (11) 0.106, (12) 0.123, (13) 0.135, (14) 0.159; (b) *cis*-[Pt(NH$_3$)$_2$(N8-Etd)Cl](OAc)$_2$ (**1a**), r_b levels for lanes 1–14, (1) 0.0, (2) 0.018, (3) 0.023, (4) 0.033, (5) 0.039, (6) 0.047, (7) 0.051, (8) 0.053, (9) 0.060, (10) 0.063, (11) 0.064, (12) 0.075, (13) 0.085, (14) 0.103; (c) *cis*-[Pt(NH$_3$)$_2$(N3-Etd)Cl](OAc)$_2$ (**1b**), r_b levels for lanes 1–14, (1) 0.0, (2) 0.030, (3) 0.045, (4) 0.051, (5) 0.053, (6) 0.058, (7) 0.061, (8) 0.064, (9) 0.065, (10) 0.068, (11) 0.069, (12) 0.071, (13) 0.075, (14) 0.077.

buffer at r_f values ranging from 0 to 1.0. After incubation for 5 h at 37 °C, the absorbance was measured at 260 nm and found not to vary as a function of the amount of bound platinum. Similarly, calf thymus DNA was treated with ethidium bromide at r_f values ranging from 0 to 0.2 and the absorbance at 260 nm monitored. Again, no effect on the absorbance as a function of added ethidium was observed.

DNA unwinding was examined by electrophoretic mobility shift assays through 0.5 mm, 1% agarose slab gels with TAE running buffer. The gels were run at 4 °C in the dark, with voltages varying between 40 and 80 V. Running time depended upon the voltage. The resultant gels were stained with ethidium bromide in water at a concentration of 0.3 μg/mL. Bands were visualized by shortwave UV transillumination and photographed on Polaroid 667 film.

Results

Electrophoresis of closed circular plasmid DNA through agarose gels can be used to monitor the degree of supercoiling. For closed circular DNA, the topology can be described by the linking number α, defined by eq 1, where β is the number of helical turns

$$\alpha = \beta + \tau \qquad (1)$$

of the DNA duplex and τ is the number of superhelical turns.[25] The linking number remains constant as long as DNA strand breakage does not occur. A compound that unwinds the DNA duplex decreases β. To keep α constant, τ, a negative number, must become more positive. The net effect is that the number of supercoils is reduced and the superhelical density decreases. This decrease in the superhelical density of closed circular DNA upon binding of unwinding agents causes a decrease in the rate of migration through agarose gels and thus provides a means by which the effect can be monitored.

Figure 2a shows an electrophoresis gel in which increasing amounts of *cis*-DDP have been bound to a mixture of nicked and closed circular pUC 19 DNA. The rate of migration of the

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supercoiled band (form I) decreases until it comigrates with the nicked relaxed band (form II), as seen in lanes 7 and 8. The bound drug-to-nucleotide ratio (r_b) at this, the coalescence, point corresponds to the amount of platinum complex that is necessary for complete removal of all supercoils from the DNA. Beyond this point, the migration rate begins to increase again as positive supercoils are induced.

From the r_b at the coalescence point, or $r_b(c)$, the unwinding angle, ϕ, can be calculated from eq 2,[25] where σ, the superhelical

$$\phi = 18\sigma/r_b(c) \qquad (2)$$

density, is a function of ionic strength and temperature. The previously determined unwinding angle of 13° for *cis*-DDP[5] and the measured r_b value were used to calculate a superhelical density of −0.055 for pUC 19 DNA under the conditions of the present experiments.

The calculation of an unwinding angle by this method requires only that the coalescence point be determined. The nature of the experiment makes this determination somewhat subjective, however, since the coalescence point is found by inspection of the gel and it is likely that none of the lanes on a particular gel will correspond precisely to that point. In an effort to minimize this problem, initial experiments were conducted over a broad range of r_b values, and from these data a much narrower range was chosen for the final determination. As can be seen from Figure 2, the r_b values near the coalescence point increase by increments smaller than 5%. In addition, for several of the compounds, the entire titration experiment was repeated 2 or more times. For these latter cases, 95% confidence limits were computed and are reported.

The unwinding gels for the two linkage isomers of *cis*-[Pt(NH₃)₂(Etd)Cl]²⁺, compounds **1a** and **1b**, respectively, are presented in parts b and c of Figure 2. These two compounds, in which platinum can form only one covalent bond with DNA, were synthesized to model the ternary complexes formed during the DNA-promoted reaction between *cis*-DDP and ethidium.[11,21] For the N8 linkage isomer **1a**, the coalescence point is 0.051 (lane 7), corresponding to an unwinding angle of 19 ± 3°, whereas the DNA molecules modified by the N3 isomer **1b** coalesce at an r_b of 0.068 (lane 10), from which a ϕ value of 15 ± 1° was computed. These unwinding angles are 9° to 13° greater than the value of 6 ± 1° measured for the monofunctional platinum complexes [Pt(NH₃)₃Cl]Cl and [Pt(dien)Cl]Cl and provide good experimental evidence for a combined intercalation/covalent binding mode. In the related complexes *cis*-[Pt(NH₃)(Etd)Cl₂]⁺, **2a** and **2b** (Figure 1), ethidium is bound to a platinum center that is also capable of binding to DNA bifunctionally, similar to the manner in which *cis*-DDP binds. When coordinated to the double helix in this fashion, the intercalator is directed away from the DNA helix. From the unwinding gels (data not shown), a ϕ value of 13° was calculated for both of these linkage isomers, the same as that for *cis*-DDP. This result indicates that the ethidium ligand does not interact with the DNA.

Similar stereochemical arguments predict that the *trans*-[Pt(NH₃)₂(N8-Etd)Cl]²⁺ complex **3** will behave in a manner analogous to the monofunctional platinum complexes **2a** and **2b**. Coordination of this compound to DNA positions the ethidium ligand trans to the binding site and directed away from the double helix. In such a binding mode there is very little contribution of ethidium to the duplex unwinding, and for the trans N8 linkage isomer **3**, ϕ = 8°. This value agrees well with the results observed for the other monofunctional platinum compounds [Pt(NH₃)₃-Cl]Cl and [Pt(dien)Cl]Cl, for which unwinding angles of 6 ± 1° have been measured. The monofunctionally coordinating complex *cis*-[Pt(NH₃)₂(4-Br(py))Cl]Cl unwinds DNA by 10°. These data and the results for all compounds studied are summarized in Table I.

The bis(platinum) complexes [{Pt(mal)(NH₃)}₂H₂N-(CH₂)₄NH₂] (**5**) and [{*trans*-PtCl(NH₃)₂}₂H₂N(CH₂)₄NH₂]Cl₂ (**6**), Figure 1, contain two *cis*-diammineplatinum(II) or two

Table I. Unwinding of Plasmid DNA by Platinum Complexes

compounds[a]	$r_b(c)$	unwinding angle, deg	reference
[Pt(NH₃)₃Cl]Cl	0.176	6 ± 1	this work
[Pt(dien)Cl]Cl	0.159	6 ± 1	this work
[{*trans*-PtCl(NH₃)₂}₂H₂N(CH₂)₄NH₂]Cl₂ (**6**)	0.145	6–7[b]	this work
trans-[Pt(NH₃)₂(N8-Etd)Cl]²⁺ (**3**)	0.125	8	this work
trans-DDP	0.11	9	this work
[Pt(NH₃)₂(4-Br-py)Cl]Cl (**4**)	0.095	10	this work
cis-DDP	0.076	13	5, 14, 15
cis-[Pt(NH₃)(N8-Etd)Cl₂]⁺ (**2a**)	0.077	13	this work
cis-[Pt(NH₃)(N3-Etd)Cl₂]⁺ (**2b**)	0.078	13 ± 1	this work
[{Pt(mal)(NH₃)}₂H₂N(CH₂)₄NH₂] (**5**)	0.075	13[b]	this work
cis-[Pt(NH₃)₂(N3-Etd)Cl]²⁺ (**1b**)	0.068	15 ± 1	this work
cis-[Pt(NH₃)₂(N8-Etd)Cl]²⁺ (**1a**)	0.051	19 ± 3	this work
[Pt{AO(CH₂)₆en}Cl₂]Cl		23 ± 3	31
ethidium bromide		26	16

[a] See Figure 1. [b] Unwinding angles are reported per platinum atom.

monofunctional platinum moieties tethered to one another by a 1,4-diaminobutane linker chain. Compounds **5** and **6** behave similarly to *cis*-DDP and [Pt(NH₃)₃Cl]Cl, respectively, producing unwinding angles of 13° and 7° per platinum (Table I). Thus, the tether, which contains no additional DNA binding functionality, does not to contribute to the DNA structural changes that accompany coordination of **5** or **6**.

Discussion

The platinum complexes evaluated in this study exhibit an extensive range of DNA unwinding angles. The values are affected by the nature of the ligands in the coordination sphere, the stereochemistry at the platinum center, and the choice of N3 or N8 linkage isomer in the case of the ethidium complexes. Table I lists the coalescence points and unwinding angles for all of the compounds arranged in order of increasing unwinding angle. Interestingly, when ordered in this way, the compounds fall naturally into classes according to their DNA binding modes. Compounds with the smallest unwinding angles (6°) are those that can bind only monofunctionally, [Pt(dien)Cl]Cl and [Pt(NH₃)₃Cl]Cl. The compound *trans*-[Pt(NH₃)₂(N8-Etd)Cl]²⁺, **3**, which unwinds DNA by 8°, can be grouped with these two monofunctional complexes. This result is readily understood in terms of an adduct structure in which ethidium, located trans to the point of attachment of platinum to a nucleobase, projects away from the DNA helix. In this orientation the intercalating moiety has little or no opportunity to interact with the double helix and thus provides no contribution to the unwinding induced by the compound. The ϕ value for this complex provides good experimental evidence for such an interpretation.

Next in the progression after the monofunctional DNA binding compounds comes *trans*-DDP. An unwinding angle of 9° has been determined for this compound. This value is significantly lower than the 13° unwinding angle observed for the biologically active cis isomer. While *cis*-DDP forms primarily 1,2-intrastrand cross-links on DNA, geometric constraints prevent *trans*-DDP from forming similar adducts. Rather, the trans isomer forms a larger family of adducts comprised primarily of long-range intrastrand and interstrand cross-links.[27–29] Bearing in mind the geometrical constraints and their effects on the adduct profiles of the two isomers, it is not surprising that the distortions they induce in DNA, as revealed by their unwinding angles, are different. Additional evidence for the different structural distortions produced by the two isomers has been provided recently. In particular, rather than forming a directed bend as does *cis*-DDP, the trans isomer forms a hinge joint, allowing the DNA helix to bend in more than one direction.[5] Conflicting results for the unwinding angle *trans*-DDP have appeared in the literature,

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however. One report found both *cis*- and *trans*-DDP to unwind DNA to the same extent,[14] whereas another determined that *trans*-DDP unwinds DNA to a lesser extent than does the cis isomer.[15,30] In both reports, gel mobility shift assays of supercoiled DNA similar to those used here were employed. The present unwinding angle falls between the two previously determined values and is in better agreement with the latter.

The next group of compounds are those for which platinum binds DNA in a bifunctional manner. Foremost in this category is the antitumor drug *cis*-DDP, which unwinds DNA by 13° as determined previously.[5,14,15] The two *cis*-[Pt(NH$_3$)(Etd)Cl$_2$]$^+$ ethidium linkage isomers, **2a** and **2b**, have the same ϕ value. This result can be interpreted in the following manner. In both cations, the platinum atom has two available coordination sites in a cis geometry and both are therefore expected to mimic *cis*-DDP with respect to their covalent attachment to DNA. As was the case for the monofunctional adducts of compound **3**, the ethidium ligand projects away from the DNA helix, with little opportunity for intercalation. Thus little or no contribution from ethidium to the unwinding would be expected, and none is observed.

The *cis*-[Pt(NH$_3$)$_2$(Etd)Cl]$^{2+}$ complexes **1a** and **1b** comprise the next category. The platinum atoms in these compounds can bind to DNA only monofunctionally. Because the ethidium is now located cis to the DNA binding site, it is geometrically well positioned to intercalate between the base pairs of the helix at the adjacent site. Previous molecular modeling studies support this combined intercalative–covalent binding mode.[11] The unwinding angles of 15° and 19° for **1b** and **1a**, respectively, are much larger than those observed for the other monofunctional compounds. This fact alone is good evidence that the ethidium ligand interacts substantially with the double helix upon covalent binding of the platinum complex, strengthening the case for a combination of intercalative and covalent binding modes. The ϕ values for **1a** and **1b** are smaller than the sum of the contributions from the two DNA binding functionalities, 26° for free ethidium plus 6° for a monofunctional platinum. This finding is not unexpected since coordination of ethidium to platinum through an exocyclic amino group will alter the geometry of its intercalation into DNA. In the case of [Pt{AO(CH$_2$)$_6$en}Cl$_2$]Cl,[31] where the intercalating and covalent binding functionalities are separated by the hexamethylene linker chain, the ϕ value of 23° more closely approximates the sum of the individual components, 30°.

Complexes **2a** and **2b** also have ethidium located cis to a DNA binding site and in principle have available a binding mode in which platinum coordinates monofunctionally while ethidium is intercalated. Perhaps, early in the time course of the reaction with DNA, these compounds do intercalate into DNA. Once the more stable bifunctional, covalent intrastrand platinum cross-link is formed, however, ethidium is oriented in a direction that is no longer compatible with significant intercalation into the DNA double helix.

A complex that shares some geometric features with **1a** and **1b** is *cis*-[Pt(NH$_3$)$_2$(4-Br(py))Cl]Cl (**4**), a monofunctional platinum compound having 4-bromopyridine coordinated cis to the site of DNA platination. Unlike ethidium, 4-bromopyridine is not a very good DNA intercalator. The large additional contribution to unwinding caused by the intercalation of ethidium in complexes **1a** and **1b** is therefore not observed for **4**. The cis geometry of the 4-bromopyridine ligand relative to the point of DNA attachment, however, forces it to interact with the DNA to some extent, and as a result **4** unwinds DNA significantly more than do the strictly monofunctionally binding complexes, [Pt(dien)Cl]Cl and [Pt(NH$_3$)$_3$Cl]Cl. An unwinding angle of 10° is observed.

One type of molecule not yet examined is that which can bind both bifunctionally at the metal center and intercalatively through a tethered ligand. Although no such complex yet exists having ethidium as the intercalator, a compound of this kind was previously synthesized in this laboratory in which acridine orange is the intercalating functionality.[31] In this compound, [Pt{AO-(CH$_2$)$_6$en}Cl]Cl, the acridine is attached to the platinum through a hexamethylene chain (Figure 1). The unwinding angle previously determined for this complex, 23°, is less than the sum of the unwinding angles of free *cis*-DDP (13°) and acridine orange (17°),[32] as mentioned above. We therefore might expect the analogous compound having ethidium as the intercalator to have an unwinding angle of greater than 26° but less than 39°. Efforts to synthesize such a molecule are in progress.

The results for [Pt{AO(CH$_2$)$_6$en}Cl]Cl further emphasize the systematic increase that occurs in DNA unwinding as one progresses from monofunctional covalent, ultimately, to bifunctional covalent binding plus intercalation. To evaluate further the applicability of this approach, the unwinding angles of the bis-(platinum) complexes [{Pt(mal)(NH$_3$)}$_2$H$_2$N(CH$_2$)$_4$NH$_2$] (**5**) and [{*trans*-PtCl(NH$_3$)$_2$}$_2$H$_2$N(CH$_2$)$_4$NH$_2$]Cl$_2$ (**6**) were measured. These complexes[18,33] are active against *cis*-DDP resistant cell lines, are recognized by Uvr ABC excinuclease, and induce a greater number of interstrand cross-links relative to those formed by *cis*-DDP.[19,20] In principle, however, the structural modifications of DNA upon binding **5** and **6** should be no different than for *cis*-DDP and [Pt(NH$_3$)$_3$Cl]Cl, respectively, since the linker chain does not introduce an additional DNA binding functionality. From these considerations one might predict that the ϕ values per bound platinum atom of **5** would be the same as that for *cis*-DDP, 13°, whereas for **6** the value should approach 6° per bound platinum atom. The observed values of 13° and 7°, respectively, confirm this expectation. It should be noted that, in a previous report,[33] it was concluded on the basis of a similar superhelical DNA unwinding study that **5** does not unwind the double helix. In that experiment, however, unwinding was monitored over a period of time at a fixed concentration, with the amount of bound platinum per nucleotide being estimated from the known r_b vs time profile of a *cis*-DDP incubation run under similar conditions. Apparently, the actual amount of bound **5** per nucleotide was not measured. Because of the relative kinetic inertness of malonate compared to chloride ligands as a leaving group, there may have been very little platinum on the DNA, which would account for the difference in the results.

Concluding Remarks

From the foregoing analysis emerges a clear correlation between binding mode and unwinding angle. In particular, there is a systematic increase in DNA unwinding as one progresses from monofunctional covalent binding through bifunctional covalent binding to monofunctional covalent binding with partial intercalation and eventually to bifunctional covalent binding with intercalation. Moreover, the measured ϕ values for the platinum–ethidium complexes provide experimental evidence for a combined covalent–intercalative DNA binding mode for **1a** and **1b**.

Many of the correlations and trends reported here, while phenomenological in nature, can be explained by the arguments presented above. Some important questions remain, however. It is not understood, for example, why the monofunctional compounds [Pt(dien)Cl]Cl and [Pt(NH$_3$)$_3$Cl]Cl unwind DNA at all nor how a particular unwinding value for any of the compounds relates to the structural distortions induced in DNA upon binding. Efforts to obtain the answers to such questions by crystallographic and spectroscopic means are currently in progress. Although the molecular details of the interactions of the complexes described here with DNA are not fully understood, the information obtained from the unwinding experiment is nonetheless valuable. In

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principle, the binding modes of other stereochemically related classes of complexes may also be able to be estimated from the unwinding angles. The potential applicability of the method to other metal and nonmetal compounds will be interesting to examine.

Stereoselective Covalent Binding of Aquaruthenium(II) Complexes to DNA

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Abstract: A group of seven mono- and diaquapolypyridyl complexes of Ru(II) have been shown to bind covalently to DNA by ultrafiltration, extensive dialysis, and ethanol precipitation. Incubation of the metal complex with calf thymus DNA in 50 mM phosphate buffer produces solutions of DNA exhibiting visible absorptions clearly due to the metal complex. These absorptions remain unchanged upon prolonged ultrafiltration or dialysis, demonstrating covalent binding of the metal complex to the DNA. Determination of the amount of bound metal complex either from the spectrum of the labeled DNA or from quantitation of the free metal complex in the filtrate obtained following ultrafiltration gives $r_b = [Ru]_b/[DNA\text{-nucleotide phosphate}] = 0.01–0.02$ for all of the complexes. Circular dichroism (CD) spectroscopy of the filtrate obtained following the reaction of DNA with racemic $Ru(phen)_2(py)OH_2^{2+}$ shows an enrichment of the solution in the Δ isomer by comparison with the known CD spectrum of the complex. Careful quantitation of the degree of enrichment in the filtrate shows that 90 $\pm$ 2% of the complexes bound to DNA are the Λ isomer, giving an enantiomeric excess for binding of the Λ isomer of 80 $\pm$ 4%. Other chiral complexes give lower selectivities, although the Λ isomer is preferred in all of the tested cases.

In studies of the interactions of metal complexes with DNA, ruthenium(II) centers can serve either as a substitution-inert template for intercalating ligands[1] or as a carrier of labile ligands that can be replaced by covalent binding to nitrogenous bases of the DNA.[2] In the latter case, early studies centered on aquapolyammine complexes,[3] and one more recent report discussed the polypyridyl complex cis-$Ru(phen)_2Cl_2$ (phen = 1,10-phenanthroline).[4] In the covalent binding of cis-$Ru(phen)_2Cl_2$, Barton and Lolis have reported a chiral selectivity that favors covalent binding of the Λ isomer to DNA. This observation is particularly striking when considered in light of the known chiral selectivity for noncovalent (intercalative) binding, which shows a preference for the opposite (Δ) isomer.

We have studied the covalent binding of an extensive series of $L_5Ru(OH_2)^{2+}$ and $L_4Ru(OH_2)_2^{2+}$ complexes in our laboratory and have obtained results consistent with the earlier findings on cis-$Ru(phen)_2Cl_2$. It has been commented[1] that the chiral selectivity for covalent binding is quite high, significantly larger than that for intercalation. To date, however, the degree of chiral selectivity for these reactions has not been carefully quantitated due to the difficulty in obtaining authentic samples of the resolved Λ and Δ isomers of the complexes studied thus far. Fortunately, a member of the series of complexes under investigation in our laboratory, cis-$Ru(phen)_2(py)OH_2^{2+}$ (Figure 1), has been resolved previously by Bosnich.[5] We report here that the covalent binding of this complex to DNA proceeds with a suprisingly high stereoselectivity.

Experimental Section

Metal Complexes. $[Ru(tpy)(bpy)OH_2](ClO_4)_2$,[6] $[Ru(tpy)(phen)OH_2](ClO_4)_2$,[7] $[Ru(tpy)(tmen)OH_2](ClO_4)_2$,[7] $[Ru(bpy)_2(py)OH_2]\text{-}$ $(ClO_4)_2$,[8] $Ru(bpy)_2Cl_2$,[9] $Ru(phen)_2Cl_2$,[4] and $Ru(bpy)_2CO_3$[9] were prepared by literature procedures (bpy = 2,2'-bipyridine, tmen = N,N,N',N'-tetramethylethylenediamine). Racemic $[Ru(phen)_2(py)OH_2](PF_6)_2$ was prepared by a method analogous to that for $Ru(bpy)_2(py)OH_2^{2+}$.[10] Anal. Calcd. for $[Ru(phen)_2(py)OH_2](PF_6)_2\cdot 2H_2O$: C, 39.43; H, 3.04; N, 7.91. Found: C, 39.44; H, 3.10; N, 8.09. UV–vis, λ, nm (ϵ, M^{-1} cm^{-1}): 466 (10100), 422 sh (10600), 318 sh (6800), 266 (85000). Preparation of Δ-$Ru(phen)_2(py)OH_2^{2+}$ by the method of Bosnich[5] gave the reported CD spectrum.

Binding Measurements. Calf thymus DNA was purchased from Sigma and used as described.[11] All experiments were performed in 50 mM phosphate buffer, pH 7. Water was obtained from a Millipore filtration system. Ethanol precipitation experiments were performed as described by Barton and Lolis.[4] Ultrafiltration was carried out in either a 3-mL or a 180-mL cell from Amicon with a 3000 molecular-weight-cutoff membrane. The results were identical using either cell. Buffer was added to the DNA compartment until no free ruthenium could be detected by optical spectroscopy in filtrate fractions. Quantitation of bound ruthenium from either the spectrum of the DNA or the spectrum of the filtrate gave identical values of r_b. Extensive dialysis was performed in a bag of 3000 molecular weight cutoff tubing (3–4 mL) against 4 L of phosphate buffer. Dialysis was continued for at least 72 h, during which time the buffer was changed 3 times. Continued dialysis showed no change in the ruthenium concentration inside the dialysis bag.

Absorption spectra were obtained by using an HP8452 diode array spectrophotometer. CD spectra were acquired on a JASCO J-600 spectrophotometer. During experiments involving chiral complexes, the acquisition of reproducible CD spectra was strongly dependent on careful protection of the samples from light. When light was not carefully excluded, lower enantiomeric excesses were obtained. Circular dichroism (CD) spectra of filtrates obtained following ultrafiltration of $Ru(phen)_2(py)OH_2^{2+}$ in the absence of DNA showed no signal, ruling out resolution of the complexes by the membrane itself.

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