Volume 13 Number 8 1985 Nucleic Acids Research

Unwinding of supercoiled DNA by cis- and trans-diamminedichloroplatinum(II): influence of the torsional strain on DNA unwinding

William M.Scovell and Frank Collart*

Department of Chemistry, Bowling Green State University, Bowling Green, OH 43403, and *Department of Biochemistry, Medical College of Ohio, Toledo, OH 43699, USA

Received 27 July 1984; Revised and Accepted 28 March 1985

ABSTRACT

The effective unwinding angle, Ø, for cis-diamminedichloroplatinum(II) (cis-DDP) and trans-DDP was determined by utilizing high resolution gel electrophoresis and supercoiled ØX174 RF DNA as a substrate. The effective unwinding angle was calculated by equating the reduction in mobility of the DDP-modified DNA to the removal of a number of superhelical turns. The value of the effective unwinding angle for both DDP isomers was greatest at the low levels of DDP bound and decreased with increasing amounts of unwinding agent. The cis-isomer is a better unwinding agent than is the trans-isomer, being nearly twice as effective in unwinding the supercoiled DNA at the DDP levels investigated. A comparison of the magnitude of Ø below r_b values of 0.005 and those at high levels of binding reveals that the extent of torsional strain in the supercoiled DNA influences the magnitude of the unwinding of the DNA by these complexes. When this method is used in the analysis of the unwinding angle for a covalently bound species on supercoiled DNA, it may provide a more reliable estimate of the magnitude of Ø at high degrees of supercoiling and at low levels of modification.

INTRODUCTION

Cis-diamminedichloroplatinum(II) is an effective antitumor drug, being especially effective in combination treatment of testicular cancer.[1-2] The trans-DDP† is inactive. It is established that both DDP isomers exhibit a preference for binding covalently to the accessible N-7 site on guanine residues in DNA.[3] Because of the different stereochemistries of the isomers and the lability of two (Pt-Cl) bonds in each case, a number of (DDP-DNA) adduct types may be anticpated for both isomers. These include intra- and interstrand DNA cross-links, in addition to those interactions involving simply monodentate binding by DDP.[4-7] It has been reported, however, that at low levels of cis-DDP binding to DNA, the intrastrand cross-link between adjacent guanines, a lesion which is stereochemically disallowed for trans-DDP, accounts for more than 50% of the adducts.[5] The unique character of particular binding modes has been revealed in studies with a number of nucleases which can differentially recognize and/or act on these sites.[6,8,9]

As with other agents which covalently modify DNA,[10,11] both DDP isomers bind to and modulate the electronic distribution in the bases, which results in a change in the orientation of the phosphodiester backbone, and thereby effects an unwinding of the DNA. By determining the amount of each DDP isomer which unwound the supercoiled DNA to the intact relaxed form, the effective unwinding angle, $\emptyset$, for cis-DDP was determined to be almost twice as large as that for trans-DDP.[12] In that report, however, the actual values for $\emptyset$ were not determined. These findings are in disagreement with those of Cohen et al.[13] who concluded that the unwinding angles for the two DDP isomers were the same. To critically examine and evaluate the unwinding angles for these two isomers and resolve this point, we have used high resolution gel electrophoresis to determine the unwinding angles for both cis- and trans-DDP on torsionally strained supercoiled DNA as a function of the amount of bound DDP. This method permits an evaluation of $\emptyset$ at low levels of covalent modification and additionally reveals that the extent of torsional strain in the DNA influences the magnitude of the effective unwinding angle obtained.

MATERIALS AND METHODS

ØX174 RF DNA was purchased from Bethesda Research Laboratories. The cis- and trans-$(NH_3)_2PtCl_2$ were generous gifts from Mathey Bishop Co. and the National Cancer Institute, respectively. Cis- and trans-DDP solutions were prepared in double-distilled H_2O by stirring at room temperature for 16-24 hours in the absence of light. The solution pH was adjusted to 5.0-6.0 with dilute NaOH and the concentration of platinum determined by atomic absorption spectrophotometry. Solutions were stored at room temperature in the dark and used within one week of preparation.

Reaction of ØX174 RF DNA with cis- and trans-DDP

Aliquots of negatively supercoiled ØX174 RF DNA containing 0.3-0.5 µg of DNA (22 µg/mL) in 3.3 mM Tris-HCl, 1.67 mM NaCl, 0.33 mM EDTA, pH 7.4, were reacted with cis- or trans-DDP with the molar ratio of DDP to DNA nucleotide, r_i, in the range of 5×10^{-4} to 1×10^{-2}. The samples were incubated at 37°C for 10 hours, followed by drop dialysis[14] (Millipore VM type membrane filters, 0.05 µm) at 25°C for one hour against 5 mM Tris-HCl, pH 8.0. Concentrated electrophoresis loading buffer (25% glycerol, 50 mM EDTA, 5% SDS, 0.025% bromophenol blue) was added to the samples and the mixtures applied to a 2% agarose gel in 40 mM Tris-HCl, 30 mM NaH_2PO_4, 1 mM EDTA, pH 7.8. Electrophoresis was carried out at 2.5 V/cm for 50-60 hours with continuous recirculation of buffer. Alternatively, the DDP-modified DNA was relaxed with topoisomerase and

the DDP removed prior to electrophoresis of the DNA. For example, after the reaction of DDP with DNA and the subsequent drop dialysis, one-tenth volume of 10x concentrated eukaryotic topoisomerase reaction buffer (250 mM Tris HCl, 250 mM KCl, 5 mM EDTA, 0.1 mM DTT, pH 7.5) was added and 1.5 units of calf thymus topoisomerase (BRL) reacted with each sample at 20°C for 30 minutes. The reaction was terminated by addition of SDS to 1%. DDP bound to DNA was then removed by incubating the samples with freshly prepared 0.2 M NaCN at 37°C for four hours. Samples were added to electrophoresis loading buffer and analyzed by electrophoresis in 1% agarose in 10 mM $Mg(C_2H_3O_2)_2$, 1 mM EDTA, 40 mM Tris acetate, pH 7.8. Gels were stained with ethidium bromide and the bands visualized on an ultraviolet light source. Photographs of the UV illuminated gel were taken using Polaroid PN55 film and a Wratten No. 9 gelatin filter. The photographic negative was scanned on a Joyce Loebl Chromoscan 200 densitometer.

Binding profiles of cis- and trans-DDP on calf thymus DNA

Preliminary studies were carried out on DDP binding to calf thymus DNA to determine the relationship between the mole ratio of DDP bound per DNA nucleotide, r_b, as a function of r_i under these conditions. Because of the relatively large sample sizes needed to acquire these data, calf thymus DNA provided a convenient and more economical source of DNA for this determination. Known quantities of calf thymus DNA were incubated with cis- or trans-DDP under the conditions described for modification of ØX174 RF DNA. After drop dialysis, the extent of DDP bound to DNA was determined by atomic absorption spectrophotometry of platinum. These studies revealed that at least 90% of the cis- or trans-isomer is covalently bound to the DNA at low r_i levels (less than 0.01). This is an approximation, however, due to the possible supercoil-dependent changes in the binding affinity. Previous studies[15,16] have shown that binding of unwinding agents to supercoiled DNA is thermodynamically favored and for several unwinding agents,[10,11,17] this may amount up to as high as a 10% increase in the level of bound molecule on supercoiled relative to relaxed DNA. Since the analogous information on DDP binding is not known, the binding correlation, $r_b = 0.9\ r_i$ reported here (data not shown) was utilized to estimate the level of bound DDP to ØX174 RF DNA in the unwinding angle studies.

Evaluation of the effective unwinding angle, Ø, for cis- and trans-DDP

Agarose gels were prepared and the fronts of the sample wells were leveled prior to the sample application and electrophoresis. The resultant electrophoretic gel pattern was photographed and a line was drawn on the photographic negative perpendicular to the direction of DNA migration. This served as a

common alignment reference for all subsequent measurements. This line was parallel to the sample well fronts and appears in Fig. 2 as a sharp (starred) peak on the densitometer scan. In the scan of unmodified DNA, the band for the most probable topoisomer species was assigned a value of '0' and the adjacent topoisomer bands were counted as positive or negative values from this reference band. Although a bimodal distribution of topoisomers occurred in this sample, only the distribution of high superhelicity was considered in these measurements. A standard plot of migration distance versus the number of supercoils for the unmodified DNA was obtained from the densitometer tracing of the gel pattern. With both untreated and DDP-modified samples on the same gel, the migration distance corresponding to the center of the topoisomer distribution in the DDP-modified DNA was measured and matched to the migration distance of a topoisomer band in the standard plot (not necessarily an integer). By equating the observed mobility difference between the center of the DDP-modified DNA distribution and the most probable species in the unmodified DNA to the change (i.e., removal) in the number of superhelical turns, the effective unwinding angle, Ø, was calculated from the equation:

$$\text{Ø} = [(\Delta\tau)(360°)/(r_b)(10{,}772\ N)]$$

in which $\Delta\tau$ is the change in the number of supercoils in the DNA as a result of DDP binding and 10,772 N is the number of nucleotide units in ØX174 RF DNA.

RESULTS AND DISCUSSION

The unwinding angle for cis- and trans-DDP was determined by a direct method utilizing ØX174 RF DNA as the substrate. This DNA is a covalently closed, circular superhelical molecule of 5386 base pairs. As shown in Fig. 1 (lanes 1, 5 and 7) the ØX174 RF DNA contains a distribution of topoisomers which is readily resolvable by the electrophoresis procedure outlined by Shure and Vinograd.[18] The distribution of topoisomers in this sample of ØX174 RF DNA is bimodal, with approximately 50% of the DNA contained in the distribution of higher superhelicity. The center of this distribution displays the most probable species (represented by the dashed line through the unmodified DNA sample in Fig. 2A and B) and is probably more accurately represented by the center of mass which need not correspond to an integral number of supercoils. However, since the distribution is symmetric, the location of the most prominent band does not differ by more than ± 0.5 supercoils from the center of mass for the distribution. Since each individual band represents a topological isomer of DNA and the adjacent bands correspond to topoisomers which differ by one supercoil, the well-resolved distribution of topoisomer bands provides the

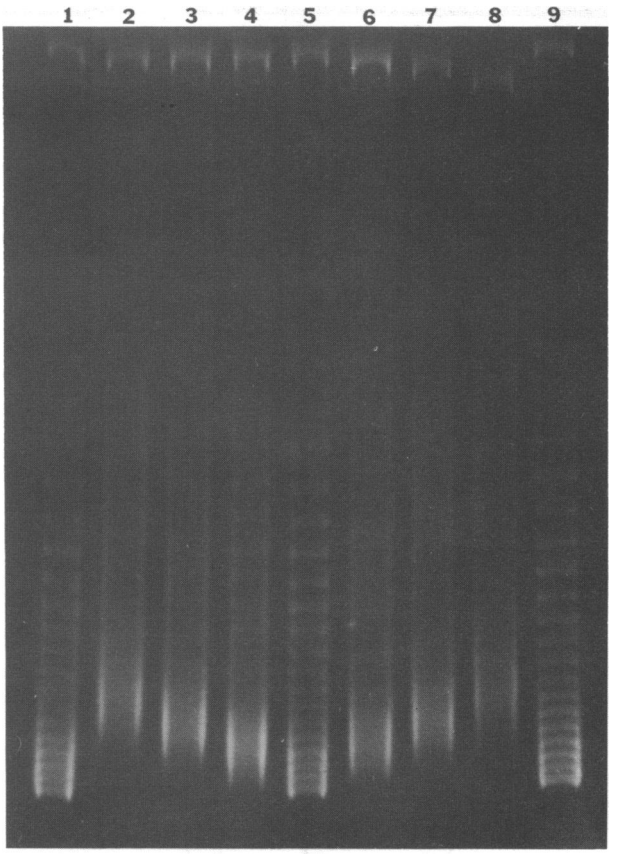

Figure 1: Agarose gel electrophoresis of cis- and trans-DDP-modified ØX174 RF DNA. Lanes 1, 5 and 9 contain unmodified DNA. The r_b values for trans-DDP modified DNA in lanes 2-4 are 0.006, 0.003, 0.001, respectively, while the r_b values for the cis-DDP modified DNA in lanes 6-8 are 0.0008, 0.002, and 0.005, respectively.

means to correlate the migration distance in the gel to the integral number of supercoils. A comparison of the migration distance of the cis-DDP modified DNA with the equivalent migration of an unmodified DNA topoisomer provides a direct means to calculate the effective unwinding angles for the DDP isomers.

As can be observed by the band positions in Fig. 1 and 2, the cis- and trans-DDP modification of ØX174 RF DNA results in a clear decrease in the electrophoretic mobility of the most highly supercoiled topoisomers, in addition to a progressive loss of resolution in the individual topoisomer bands with increasing levels of bound DDP. This is observed at all r_b levels investi-

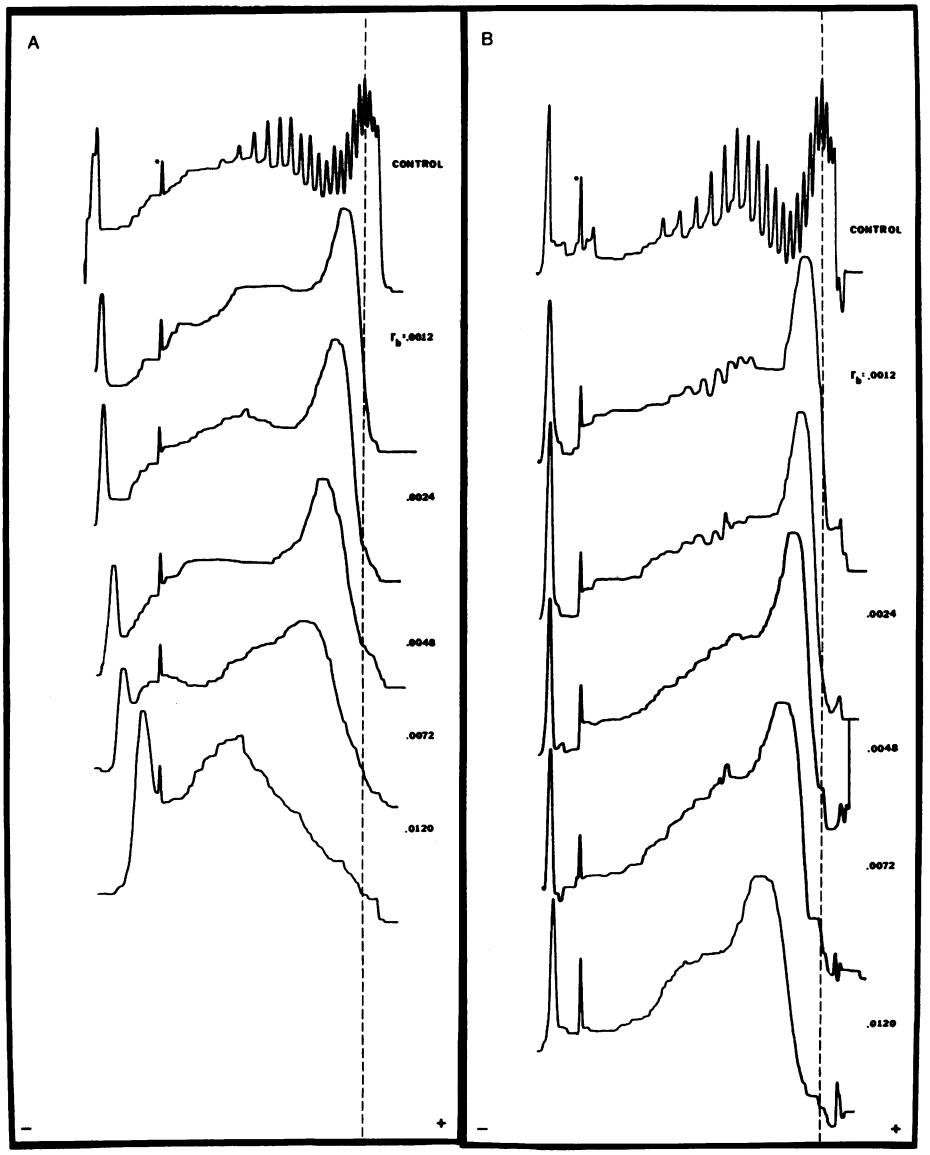

Figure 2: Densitometric tracings of the band patterns for DDP-modified ØX174 RF DNA. The exact alignment permits the calculation of the average number of superhelical turns removed by (A) cis-DDP and (B) trans-DDP binding as a function of the r_b. The dashed line indicates the migration distance of the most probable species in unmodified DNA. The starred (*) peak results from a line drawn on the photographic negative which was utilized as a common alignment point.

gated, including r_b = 0.0008, which corresponds to less than 10 cis-DDP/genome. The decreased electrophoretic mobility is similar to that which has been observed when agents[10-12] unwind supercoiled DNA. In previous studies with cis- and trans-DDP binding to supercoiled SV40 DNA, both isomers were observed to decrease the electrophoretic mobility of supercoiled DNA and at relatively high levels of cis- and trans-DDP bound (r_b = 0.08 and 0.15, respectively), the mobility of the modified DNA decreased to that of the intact relaxed form.[12] At higher r_b values of cis-DDP, the electrophoretic mobility increased consistent with the formation of positive supercoils. This effect was not observed with trans-DDP. These results[10-12] further support the interpretation that the decrease in electrophoretic mobility of DDP-modified DNA observed at the very low r_b levels in this study results from cis- and trans-DDP acting as an unwinding agent of negatively supercoiled DNA.

There are several factors which may contribute to the loss of resolution of the individual topoisomers. One of these is the formation of a distribution of DDP adducts on each topoisomer which produces virtually a continuous set of conformational isomers for DNA. The nature of these topoisomers will differ by the magnitude of the DDP unwinding angle and are therefore not resolved by electrophoresis. The effects of the DDP modifications may also be influenced by a DNA sequence dependence[4,19] and the resultant distribution of the various modes of DDP binding, each of which will influence the magnitude of the unwinding angle. These effects increase the number of possible conformations imposed on each topoisomer and thereby broaden the distribution.

Cis-DDP binding to nicked (form II) DNA increases its electrophoretic mobility (Fig. 1 and 2), while little or no increase is observed for trans-DDP modified DNA at these low r_b levels. The increased mobility of the modified-nicked DNA has been noted in previous studies with both the cis- and trans-DDP isomers[12] and benzo[a]pyrene-diol-epoxide.[11] This effect is thought to result from a decrease in the length of the DNA attributable to local denaturation or microloop formation,[13,20] although this may be controversial at these low r_b levels.[8] In addition, there is essentially no evidence for any degree of strand scission occurring during the reactions.

The magnitude of the effective unwinding angle was determined by counting the number of topoisomer bands between the most probable species in the unmodified DNA and the center of the DDP-modified distribution. The analysis utilized only the DDP-modified DNA in the highly supercoiled distribution. The point of maximum intensity in the band associated with the modified DNA was utilized to determine the number of supercoils removed. This method for deter-

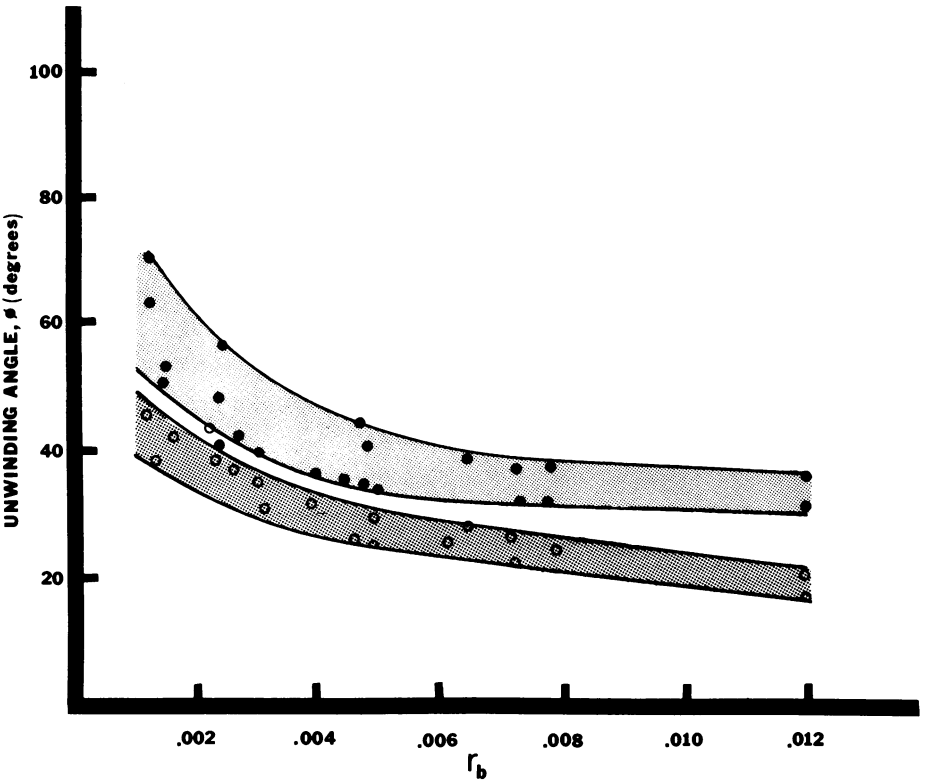

Figure 3: Calculated effective unwinding angle for cis- (●) and trans-DDP (○) on DDP-modified ØX174 RF DNA as a function of r_b, with bands enveloping values obtained from different determinations.

mining the unwinding angle necessitates a higher uncertainty in the value of ϕ at the lowest r_b levels. This uncertainty is, of course, attributed to the smaller mobility changes which are observed at these r_b levels. To minimize these factors, the center well containing the unmodified DNA was adjacent to the lowest r_b level of both DDP-modified DNAs (Fig. 1) and was used in the determination of the unwinding angle. The outermost wells contained unmodified DNA to insure reproducibility of the gel pattern. In addition, the unwinding angles were not determined for r_b levels greater than 0.012 due to the increasing significance of factors other than unwinding of the DNA affecting the electrophoretic mobility.

A summary of the values for the unwinding angle, as a function of r_b for both cis- and trans-DDP, is displayed in Fig. 3. As is evident, particularly

at low r_b values, the magnitude of the average unwinding angle for both cis- and trans-DDP depends on the amount of DDP bound (i.e., the r_b value). The value of Ø for each DDP isomer is greatest at the lower r_b values, with the effective unwinding angle decreasing with increased r_b value. For the cis-DDP isomer, the unwinding angle is ca. 35° at r_b = 0.01, while at lower levels of binding, the value of Ø increases, exceeding a value of 60° at r_b = 0.001. Similarly, the unwinding angle for trans-DDP is larger at the lower r_b values. At r_b = 0.01, Ø is nearly 20°, while at r_b = 0.001, Ø is more than 40°.

Interestingly, previous findings[12] indicate that the intact relaxed form of SV40 DNA is observed at r_b levels for cis- and trans-DDP of 0.08 and 0.15, respectively. If it is assumed that the SV40 DNA contained 26 supercoils,[18] the average unwinding angle for cis- and trans-DDP calculated at the point of these much larger r_b values would be 11 and 6 degrees, respectively. These values, however approximate, are significantly lower than those reported here, but are consistent with the trend of decreasing average unwinding angles with increasing r_b. Over this extended range of r_b values, the values for Ø for both DDP isomers change significantly. At virtually all r_b values, except at the very lowest ones, cis-DDP is nearly twice as effective an unwinding agent at the trans-isomer.

The influence that the intrinsic torsional strain in supercoiled DNA exerts on the magnitude of the effective unwinding angle was examined. This was examined by determining the unwinding angle for the DDP isomers at r_b levels < 0.010 and on the DDP-modified DNA which was relaxed after the reaction. This Ø value was then compared to our findings on the DDP-modified DNA which was torsionally strained. Experimentally, the supercoiled ØX174 RF DNA was modified with cis- or trans-DDP as described previously and then relaxed with calf thymus eukaryotic Type I topoisomerase. Figure 4 shows the topoisomer distribution for the relaxed DNAs after removal of bound drug by incubation with 0.2 M NaCN. The average shift in the migration distance of the resolved topoisomer bands is used to determine the number of superhelical turns restored to the DNA and the corresponding value of Ø. Specifically, values of Ø were determined at r_b levels from 0.001 to 0.007 for both cis- and trans-DDP. On the topoisomerase relaxed substrate, an unwinding angle of 12° was determined for cis-DDP at all r_b levels. Similarly, the Ø value of 6° was obtained for trans-DDP. It is striking that these values are not only so much lower than the corresponding values determined on the supercoiled substrate, but that they are independent of r_b in this range and in agreement with the previous estimate of Ø on DNA in the intact relaxed form (Table I and text). This clearly indicates that in the

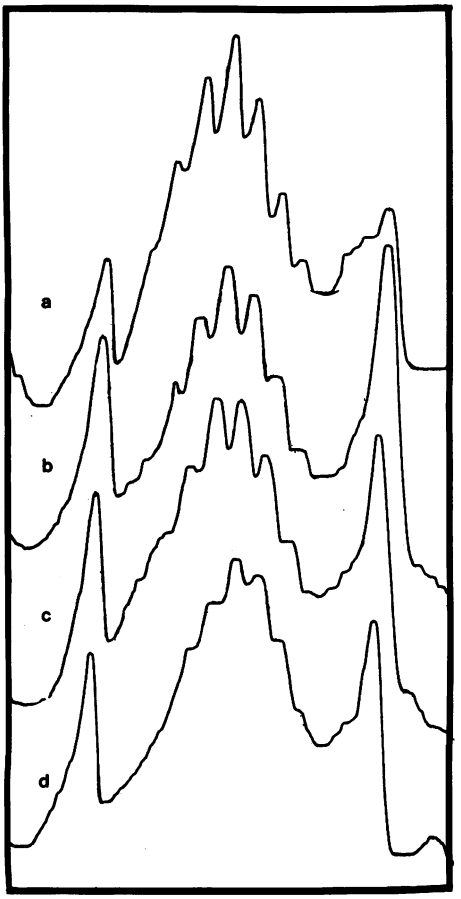

Figure 4: Densitometer tracings of the electrophoretic pattern resulting from the relaxation of ØX174 RF DNA containing bound cis-DDP at r_b levels of 0.001, 0.002 and 0.004 (tracings b-d, respectively). The bound cis-DDP was removed with 0.2M NaCN prior to electrophoresis. Unmodified DNA relaxed by calf thymus topoisomerase I is shown in tracing a.

absence of torsional strain, the unwinding angle for either DDP isomer is considerably reduced from that observed at a low level of binding on the highly supercoiled DNA. Table 1 summarizes the unwinding angles for both DDP isomers and reveals the trend in Ø values, extending over two orders of magnitude of

Table 1: Unwinding Angles for Cis- and Trans-DDP as a Function of Amount Bound (r_b).

r_b	DDP / genome	Ø	
		cis-DDP	trans-DDP
0.0008	8	> 60°	> 45°
0.008	80	40°	25°
0.08	800	11°	6°

r_b. In addition to this, the DDP unwinding angles obtained at low r_b values on the topoisomerase relaxed DNA are essentially the same as those calculated at high r_b on the relaxed intact form of DNA. Both these results provide additional support that torsional strain in DNA influences the effective Ø value observed.

In this regard, it is important to emphasize that these findings are consistent with an unwinding mechanism in which the torsional strain intrinsic to highly supercoiled DNA enhances the ability of DDP binding to remove supercoils from the DNA. The number of supercoils, τ, in the DNA is equal to the topological linking number difference,[21] $\Delta\alpha = (\alpha - \alpha^\circ)$, in which α is the topological linking number which defines the degree of intertwining of the two complementary DNA strands in covalently closed circular DNA and α° is the linking number for the same DNA in the absence of any torsional stress. With the binding of the first few molecules of DDP to highly supercoiled DNA, it appears that unwinding is more effective due to the strain induced by a large number of supercoils. This binding results in an unusually large reduction in the number of supercoils and a large value for the unwinding angle. DDP binding does not change the topological linking number, α, of the DNA since the phosphodiester backbone remains intact. However, since the number of superhelical turns is reduced, DDP binding changes $\Delta\alpha$ for the DNA by decreasing the effective value of $\underline{\alpha^\circ}$. Since the free energy associated with supercoiling is described by the equation, $\Delta G = K\,\tau^2 = K\,(\Delta\alpha)^2$, the free energy of supercoiling is expected to decrease exponentially as increasing amounts of DDP bind to the DNA. This results in a progressive decrease in torsional strain in the DNA, a correspondingly smaller influence on further DDP mediated unwinding and the resultant lower unwinding angle at higher r_b values.

Table 2 lists the values of the unwinding angle for a number of DNA modifying agents. Those studies which determined the unwinding angle using a supercoiled DNA substrate (i.e., not relaxed by topoisomerase I prior to determining Ø) are marked with an asterisk (*). It can be seen that the values of Ø for many small molecules range from 6-30 degrees. All of these values were obtained under conditions of little or no torsional strain. In the three cases in which the value of Ø was also determined directly on a DNA exhibiting high torsional strain, larger values for the unwinding angle were obtained. Note that in addition to our findings with cis- and trans-DDP, the Ø values for benzo[a]pyrene-diol-epoxide also exhibit this same dependence on the torsional stress and the r_b value.[11] The methodology used here to determine the effective unwinding angle of cis- and trans-DDP should be generally applicable to

Table 2: Unwinding Angles for Some Agents Which Modify DNA

Agent	Ø degrees/[agent]	Reference
Ethidium bromide	26	22
N-acetoxy-2-acetylaminofluorene (AAAF)	22	23
Benzo[a]pyrene-diol-epoxide (BP)	13	24
	22†	23
	30-330*	11
4,5',8-trimethylpsoralen	28	25
Bleomycin	12	10
Cis-$(NH_3)_2PtCl_2$	11†	this work
	12	this work
	40-60*	this work
Trans-$(NH_3)_2PtCl_2$	6†	this work
	6	this work
	20-45*	this work
E. coli RNA polymerase	580	26
	240	27
Pyrimidine dimer	6	28
	14	29
Daunomycin	15	30

* Values determined on highly supercoiled DNA

† Value calculated from data in reference

other covalently bound DNA-modifying agents and perhaps to other non-covalently bound agents. It may be expected that the magnitude of the unwinding angle for agents which bind covalently would be influenced by the extent of DNA superhelicity and that these agents would exhibit greater unwinding angles at higher levels of torsional strain.

The findings of this study agree with previous reports[12] which clearly indicate that the unwinding angle for the cis- and the trans-DDP are different. The average difference for the two isomers at r_b = 0.01 is nearly 20 degrees, with the unwinding angle for cis-DDP being nearly twice that for trans-DDP. These results support the general finding of Scovell and Kroos[12] that cis-DDP is about twice as effective an unwinding agent as the trans-DDP isomer. In addition, they are also consistent with the experimental observation that at the same r_b level, the resolution of the individual topoisomers bands is decreased more by the cis- than the trans-isomer. As a result of the numerous possible modes of DDP binding to DNA, the calculated unwinding angle must be representative of a weighted average of the unwinding contributions from each mode in this ensemble. Binding to N-7 of guanine is expected to be the predominant site of modification but the unwinding angle will also be influenced significantly by the mode of binding and the superhelicity dependent effects already pointed out. The modes of binding include a number of possible biden-

tate binding modes, especially the intrastrand binding to adjacent guanines at N-7 which is only sterically accessible to the cis-DDP isomer. Recent results obtained by Eastman[5] on cis-dichloro(ethylenediammine)platinum(II) (an analogue of cis-DDP) show that at low levels of DNA modification, over half of the lesions involve the intrastrand cross-link between two adjacent guanines. For cis-DDP, this mode of bonding has been suggested to result in a "kink" in the DNA helix[31] and therefore could result in substantial unwinding of the DNA. At higher levels of binding, the contribution of this mode of binding to the total will become progressively "diluted," in line with the progressive reduction in the magnitude of $\emptyset$ with increasing r_b. Although this may occur, it does not explain, of course, the very different unwinding angles observed for cis-DDP on the superhelical and the (topoisomerase) relaxed DNAs since the initial DDP binding in both cases occurs on the highly supercoiled DNA. This again prompts one to invoke torsional strain as influencing the magnitude of $\emptyset$ observed. One may consider that each lesion can unwind DNA to a certain degree and as such, provide a nucleation site for further local denaturation. This denaturation would be expected to be most pronounced at high superhelicity since it too provides a route to relieve torsional strain by changing $\alpha°$, just as does the actual DDP unwinding of the DNA backbone. It would not be surprising if this were true for both DDP isomers to different extents and to other DNA modifying agents in general.

Interestingly, the influence of torsional stress in superhelical DNA on the magnitude of $\emptyset$ parallels many well-established supercoiled-driven phenomena such as S1 cleavage specificity,[32-34] nuclease hypersensitivity,[35] RNA polymerase binding to DNA,[36] cruciform formation[32,33] and the B to Z form transition in DNA.[21] From a thermodynamic standpoint, however, it would appear that the B to Z form DNA transition is unlikely to contribute to the effective unwinding angle. For example, the decrease in ΔG which results from DDP unwinding the DNA is analogous to the unwinding that also occurs in the B to Z-DNA transition. In both cases, the resultant decrease in superhelicity and the corresponding decrease in ΔG derive directly from a decrease in $\alpha°$ and its relationship to ΔG, since $\Delta G = K (\alpha - \alpha°)^2$. Previous studies have suggested that modification of certain DNA sequences by derivatives of AAAF[37] or aflatoxin B1 could influence the B to Z conformational transition. Similar investigations with platinum containing complexes, including cis-DDP have presented conflicting findings.[39-41] In light of these contradictory studies, it should be recalled that the potential for segments of DNA to undergo the B to Z transition is enhanced by a high superhelical density in the DNA.[42,43]

Therefore, DDP binding which unwinds the DNA and reduces the superhelicity and the concomitant free energy as a result of decreasing α° would be expected to significantly lower the potential for a B to Z-DNA structural transition. It follows that from these thermodynamic considerations alone, DDP binding would not be expected to enhance the potential for a B --> Z DNA transition. Therefore, the larger Ø values for the DDP isomers at high superhelicity are probably not associated with a segment of DNA undergoing a B ----> Z form transition.

†Abbreviations: Cis- and trans-DDP, cis- and trans-diamminedichloroplatinum(II); r_b, the moles of DDP bound per DNA nucleotide; r_f, the moles of DDP reacted per DNA nucleotide; Ø, unwinding angle; N, nucleotide; τ, number of supercoils; $\Delta\tau$, change in the number of supercoils; α, topological linking number in covalently closed circular DNA; α°, linking number in the absence of torsional stress; $\Delta\alpha$, = $(\alpha-\alpha^\circ)$.

REFERENCES

1. Einhorn, L.H. (1981) Cancer Res. 41, 3275-3280.
2. Carter, S.K. (1984) in Platinum Coordination Complexes in Cancer Chemotherapy, Hacker, M.P., Douple, E.B. and Krakoff, I.H. Eds., pp. 359-376, Martinas Nijhuff Publishing, Boston.
3. Martin, R.B. (1983) in Platinum, Gold and Other Metal Chemotherapeutic Agents, Lippard, S.J. Ed., ACS, Symposium Series #209, 231, Washington.
4. Royer-Pokora, B., Gordon, L.K. and Haseltine, W.A. (1981) Nucleic Acids Res. 9, 4595-4609.
5. Eastman, A. (1983) Biochemistry 22, 3927-3933.
6. Tullius, T.D. and Lippard, S.J. (1981) J. Am. Chem. Soc. 103, 4620-4622.
7. Zwelling, L.A., Kohn, K.W., Ross, W.E., Ewig, R.A.C. and Anderson, T. (1978) Cancer Res. 38, 1762-1786.
8. Scovell, W.M. and Capponi, V.J. (1982) Biochem. Biophys. Res. Commun. 107, 1138-1143.
9. Scovell, W.M. and Kroos, L.R. (1982) Biochem. Biophys. Res. Commun. 108, 16-23.
10. Povirk, L.F., Hogan, M. and Dattagupta, N. (1979) Biochemistry, 18, 96-101.
11. Gamper, H.B., Straub, K., Calvin, M. and Bartholomew, J.C. (1980) Proc. Natl. Acad. Sci., USA 77, 2000-2004.
12. Scovell, W.M. and Kroos, L.R. (1982) Biochem. Biophys. Res. Commun. 104, 1597-1603.
13. Cohen, G.L., Bauer, W.R., Barton, J.K. and Lippard, S.J. (1979) Science 203, 1014-1016.
14. Murusyk, R. and Sergeant, A. (1980) Anal. Biochem. 105, 403-404.
15. Bauer, W. and Vinograd, J. (1968) J. Mol. Biol. 33, 141-171.
16. Hsieh, T. and Wang, J.C. (1975) Biochemistry 14, 527-535.
17. Hyde, J.E. and Hearst, J.E. (1978) Biochemistry 17, 1251-1257.
18. Shure, M. and Vinograd, J. (1976) Cell 8, 215-226.
19. Scovell, W.M. and Capponi, V.J., in preparation.
20. Macquet, J.P. and Butour, J.L. (1978) Biochimie 60, 901-914.
21. Nordheim, A., Lafer, E.M., Peck, L.J., Wang, J.C., Stollar, B.D. and Rich, A. (1982) Cell 31, 309-318.
22. Wang, J.C. (1974) J. Mol. Biol. 89, 783-801.

23. Drinkwater, N.R., Miller, J.A., Miller, E.C. and Yang, N.-C. (1978) Cancer Res. 38, 3247-3255.
24. Meehan, T., Gamper, H., Becker, J.F. (1982) J. Biol. Chem. 257, 10479-10485.
25. Wiesehahn, G. and Hearst, J.E. (1978) Proc. Natl. Acad. Sci., USA 75, 2703-2707.
26. Gamper, H.B. and Hearst, J.E. (1982) Cell 29, 81-90.
27. Wang, J.C., Jacobsen, J.H. and Saucier, J.-M. (1977) Nucleic Acids Res. 4, 1225-1241.
28. Camerman, N. and Camerman, A. (1968) Science 160, 1451-1452.
29. Ciarrocchi, G. and Pedrini, A.M. (1982) J. Mol. Biol. 155, 177-183.
30. Fritzsche, H., Triebel, H., Chaires, J.B., Dattagupta, N. and Crothers, D.M. (1982) Biochemistry 21, 3940-3946.
31. den Hartog, J.H.J., Altona, C., Chottard, J.C., Girault, J.P., Lallemand, J.Y., de Leeuw, F.A.A.M., Marcelis, A.T.M. and Reedijk, J. (1982) Nucleic Acids Res. 9, 4715-4730.
32. Lilley, D.M.J. (1980) Proc. Natl. Acad. Sci., USA 77, 6468-6472.
33. Panayotatos, N. and Wells, R.D. (1981) Nature 289, 466-470.
34. Wang, J.C. (1974) J. Mol. Biol. 87, 797-816.
35. Weintraub, H. (1983) Cell 32, 1191-1203.
36. Botchan, P., Wang, J.C. and Echols, H. (1973) Proc. Natl. Acad. Sci., USA 70, 3077-3081.
37. Wells, R.D., Miglietta, J.J., Klysik, J., Larson, J.E., Stirdivant, S.M. and Zacharias, W. (1982) J. Biol. Chem. 257, 10166-10171.
38. Nordheim, A., Hao, W.M., Wogan, G.N. and Rich, A. (1983) Science 219, 1434-1436.
39. Malfoy, B., Hartmann, B. and Leng, M. (1981) Nucleic Acids Res. 9, 5659-5669.
40. Ushay, H.M., Santella, R.M., Caradonna, J.P., Grunberger, D. and Lippard, S.J. (1982) Nucleic Acids Res. 10, 3573-3588.
41. Malinge, J.-M. and Leng, M. (1984) EMBO J. 3, 1273-1279.
42. Singleton, C.K., Klysik, J., Stirdviant, S.M. and Wells, R.D. (1982) Nature 299, 312-316.
43. Peck, L.J., Nordheim, A., Rich, A. and Wang, J.C. (1982) Proc. Natl. Acad. Sci., USA 79, 4560-4564.