Binding of *cis*- and *trans*-Diamminedichloroplatinum(II) to Deoxyribonucleic Acid Exposes Nucleosides As Measured Immunochemically with Anti-Nucleoside Antibodies†

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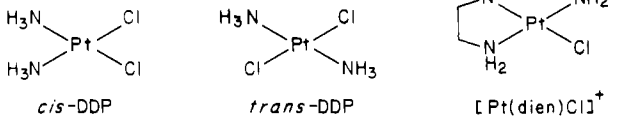
ABSTRACT: We report the use of anti-nucleoside antibodies to probe for local denaturation of calf thymus DNA upon binding of the antitumor drug *cis*-diamminedichloroplatinum(II), *cis*-DDP, and the biologically inactive analogues *trans*-diamminedichloroplatinum(II), *trans*-DDP, and chloro(diethylenetriamine)platinum(II) chloride, [Pt(dien)Cl]Cl. These antibodies specifically recognize each of the four DNA nucleosides. They bind well to denatured DNA, but not to native DNA in which the bases are less accessible owing to Watson–Crick duplex structure. At relatively high levels of modification (D/N $\sim$ 0.1), *cis*-DDP causes significant disruption of DNA base pairing as reflected by the increased binding of anti-cytidine, anti-adenosine, and anti-thymidine antibodies. At lower levels of platinum adduct formation, however, all four anti-nucleoside antibodies bind more to DNA modified with *trans*-DDP. This result indicates that adducts formed by *trans*-DDP disrupt the DNA structure to a greater extent than those formed by *cis*-DDP at low D/N ratios. Modification of DNA by the monofunctional complex [Pt(dien)Cl]Cl does not affect its recognition by anti-nucleoside antibodies, demonstrating that base pair disruption is a consequence of bifunctional binding. The relative anti-nucleoside antibody recognition of *cis*-DDP-modified DNA is anti-cytosine > anti-adenosine $\sim$ anti-thymidine $\gg$ anti-guanosine, consistent with the major adduct being an intrastrand d(GpG) cross-link. These results reveal that base pair disruption in a naturally occurring DNA modified by either *cis*-DDP or *trans*-DDP is sufficient to be detected by protein (antibody) binding. The relevance of these findings to current ideas about the molecular mechanism of action of *cis*-DDP is discussed.

The platinum complex *cis*-diamminedichloroplatinum(II) (*cis*-DDP)[1] is a highly active and clinically important antitumor drug (Hacker et al., 1984). Aspects of the mechanism of its cytotoxicity are beginning to emerge (Roberts & Pera, 1983; Ciccarelli et al., 1985). Interest has focused on the details of DNA structure modification induced by *cis*-DDP, especially in comparison to the biologically inactive trans isomer.



A number of studies suggest that normal DNA duplex structure is perturbed and that Watson–Crick base pairing is weakened or disrupted by *cis*-DDP binding (Macquet & Butour, 1978; Cohen et al., 1979, 1980; Inagaki & Kidani, 1980; Royer-Pokora et al., 1981; Tullius & Lippard, 1981; Ushay et al., 1981; Houssier et al., 1983; Scovell & Caponni, 1984). Recent NMR spectroscopic studies on *cis*-DDP-modified oligonucleotides, however, indicate that although structural changes may take place, there may be little or no base pair disruption (den Hartog et al., 1984; Van Hemelryck et al., 1984). Molecular mechanics calculations also reveal that retention of interstrand hydrogen bonding is possible, although perhaps with some modification of normal Watson–Crick hydrogen bonding (Kozelka et al., 1985). The extent of base pair disruption brought about by *cis*-DDP binding to DNA thus remains controversial and is addressed in the present study.

Changes in DNA structure upon covalent binding of the biologically inactive analogues *trans*-diamminedichloroplatinum(II) (*trans*-DDP) and chloro(diethylenetriamine)-platinum(II) chloride [[Pt(dien)Cl]Cl] have been less well investigated. From spectroscopic data, the bifunctional *trans*-DDP complex would appear to disrupt the DNA helix to a much greater extent than the monofunctional [Pt-(dien)Cl]Cl (Macquet & Butour, 1978; Inagaki & Kidani, 1980; Houssier et al., 1983; Polissiou et al., 1985). Consistent with this interpretation are replication mapping studies that show that *trans*-DDP, like *cis*-DDP, inhibits DNA synthesis by DNA polymerase I while [Pt(dien)Cl]Cl does not (Pinto

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[1] Abbreviations: *cis*-DDP, *cis*-diamminedichloroplatinum(II); *trans*-DDP, *trans*-diamminedichloroplatinum(II); dien, diethylenetriamine; D/N, bound drug-to-nucleotide ratio; *cis*-DDP– and *trans*-DDP–DNA, DNA containing bound *cis*-DDP or *trans*-DDP at a specified D/N; Tris, tris(hydroxymethyl)aminomethane; TBS, 10 mM Tris and 150 mM sodium chloride buffer, pH 7.2; PBS, 10 mM sodium phosphate and 150 mM sodium chloride buffer, pH 7.4; BSA, bovine serum albumin; EDTA, sodium salt of ethylenediaminetetraacetate; HPLC, high-performance liquid chromatography; ELISA, enzyme-linked immunosorbent assay; IgG, immunoglobulin G.

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& Lippard, 1985). Since in vivo studies show that adducts of *trans*-DDP with DNA are recognized and repaired more efficiently by the cell than those of *cis*-DDP (Ciccarelli et al., 1985), details of the differences in binding are of considerable interest. The present experiments also address this point.

Immunological methods have previously been used to probe the structure of modified nucleic acids (Poirier, 1981; Poirier et al., 1982; Lippard et al., 1983; Strickland & Boyle, 1984). In particular, antibodies that bind specifically to each of the four bases in DNA have been used to detect local denaturation brought about by UV-induced thymine dimerization (Schreck et al., 1974; Seaman et al., 1968a) and by guanine photooxidation (Seaman et al., 1968b). These studies take advantage of the fact that antibodies raised against protein-conjugated nucleosides bind well to denatured DNA but not to native DNA in which the bases are less accessible owing to their hydrogen bonding in the double helix. In addition to their relatively high sensitivity, anti-nucleoside antibodies provide insight into the base specificity of DNA modification by a chemical agent. We report here the use of anti-nucleoside antibodies to probe the extent and specificity of base pair disruption in the vicinity of platinum adducts formed on calf thymus DNA in vitro.

Materials and Methods

Anti-Nucleoside Antibodies. Rabbit antisera were previously prepared (Sandberg & Stollar, 1966; Hughes et al., 1973) by immunizing with ribonucleoside–protein conjugates (Erlanger & Beiser, 1964). Each of the antisera reacted specifically with the base against which it was raised; deoxyribonucleosides reacted somewhat better than ribonucleosides (Stollar, 1980). Serum titrations were performed, and anti-nucleoside antibodies were used at the following dilutions: anti-cytidine, 1:1000; anti-guanosine, 1:400; anti-thymidine, 1:200; anti-adenosine 1:200; all in 10 mM sodium phosphate and 150 mM sodium chloride buffer, pH 7.4 (PBS).

Platinum-Modified Nucleic Acids. *cis*-DDP, *trans*-DDP, and [Pt(dien)Cl]Cl were generous gifts from Drs. C. M. Merkel and P. K. Mascharak of our laboratory. Calf thymus DNA (Sigma) was extracted 3 times with chloroform/isoamyl alcohol (24:1) to remove any protein contaminants, precipitated twice with ethanol, and resuspended in 1 mM sodium phosphate and 3 mM sodium chloride buffer, pH 7.4, for reaction with the platinum complexes. Where noted, DNA was heat denatured by boiling for 5 min followed by rapid cooling at 4 °C. DNA samples were incubated for 24 h at 37 °C with the appropriate concentration of platinum reagent. Unreacted platinum was removed by dialysis against reaction buffer followed by 10 mM Tris and 150 mM sodium chloride buffer, pH 7.2 (TBS). Platinum drug-to-nucleotide ratios (D/N) were determined by atomic absorption spectroscopy (Ushay et al., 1981).

The triethylammonium salt of $d(GpG)^-$, prepared by the solution phosphotriester method (Sproat & Gait, 1984), was provided by S. E. Sherman. The product was characterized by NMR spectroscopy and purified by reverse-phase HPLC. *cis*-$[Pt(NH_3)_2\{d(GpG)\}]^+$ was prepared by incubating $d(GpG)^-$ with 1 equiv of *cis*-DDP in H_2O for 3 days at 37 °C. The resulting single product was purified by anion-exchange HPLC and had a measured D/N of 0.53. Previous work has shown that platinum binds to the N(7) positions of both guanosine nucleosides in this adduct (Girault et al., 1982).

ELISA (Enzyme-Linked Immunosorbent Assay). ELISAs were performed on Immulon I (Dynatech) flat-bottomed polystyrene microtiter plates as previously described (Kanai et al., 1982). The following incubation steps were carried out sequentially in the microtiter plate wells with solution volumes of 0.15 mL. Intervening washes with TBS and PBS–0.2% Tween 20 were used to remove unbound reagents. Poly(L-lysine) (50 μg/mL in H_2O) was incubated in the wells for 30 min to enhance DNA binding before DNA samples (2.5 μg/mL in TBS) were incubated for 60 min. The wells were blocked with poly(L-glutamate) (50 μg/mL in TBS, 15 h at 4 °C) and BSA (1% in 60 mM sodium phosphate and 3 mM EDTA buffer, pH 8.0) to minimize nonspecific antibody binding. Anti-nucleoside antibodies were diluted in PBS and incubated in the wells for 60 min. For competitive assays, the antibodies were preincubated with the competitors for 60 min before addition to the wells. Bound antibody was detected by incubation with a goat anti-rabbit IgG conjugated to alkaline phosphatase (1:1000 in PBS, 90 min) followed by addition of the alkaline phosphatase substrate *p*-nitrophenyl phosphate (1 mg/mL in 50 mM sodium carbonate and 2 mM magnesium chloride buffer, pH 9.5). The formation of *p*-nitrophenol was measured by its absorbance at 405 nm with a Microelisa MR580 autoreader (Dynatech); reported values are absorbance after 60 min.

Results

Anti-Nucleoside Antibody Binding to DNA Modified with cis-DDP, trans-DDP, and [Pt(dien)Cl]Cl. As observed previously (Stollar, 1980), the anti-nucleoside antibodies used in this study bind well to denatured but not native DNA. Under our ELISA conditions the absorbance resulting from antibody binding to native DNA was only slightly above background absorbance levels for nonimmune rabbit serum and in all cases was less than 15% of the absorbance resulting from antibody binding to heat-denatured DNA. The antibodies, therefore, could be used to measure changes in base accessibility upon modification of DNA with the platinum compounds.

To test for changes in anti-nucleoside antibody binding, DNA samples modified with increasing levels of the three platinum compounds were immobilized and incubated with each of the four anti-nucleoside antisera and, in control experiments, with nonimmune rabbit serum. Modification of DNA with *cis*- and *trans*-DDP increased the binding of the anti-nucleoside antibodies (Figure 1). Increases in anti-nucleoside antibody binding to *cis*-DDP-modified DNA were nonlinear and base specific. There was no significant increase in binding of the anti-guanosine antibodies to DNA modified by *cis*-DDP whereas antibodies specific for adenosine, thymidine, and cytidine showed increased binding at higher *cis*-DDP modification levels. The anti-cytidine antibody exhibited the largest increase in recognition. The binding of these three antibodies was small at low D/N but rose quickly at higher levels of modification, suggesting that *cis*-DDP induces cooperative weakening or disruption of DNA base pairing.

At low levels of *trans*-DDP modification, D/N $\sim$ 0–0.04, the binding of all four anti-nucleoside antibodies, as measured in the ELISA, increased in a linear fashion (correlation coefficients were >0.985) with increasing D/N (Figure 1). Furthermore, all four antibodies bound more to DNA modified with *trans*-DDP than to DNA modified with the same D/N of *cis*-DDP. At higher levels of *trans*-DDP modification (D/N > 0.06), antibody binding remained relatively constant with the anti-guanosine antibody binding curve reproducibly leveling off at D/N $\sim$ 0.04.

In contrast to the results obtained for *cis*- and *trans*-DDP, there was no change in anti-nucleoside antibody binding to DNA modified with [Pt(dien)Cl]Cl up to a D/N of 0.12 (data not shown). Similarly, no significant increase in the low background level of nonimmune serum binding to DNA was

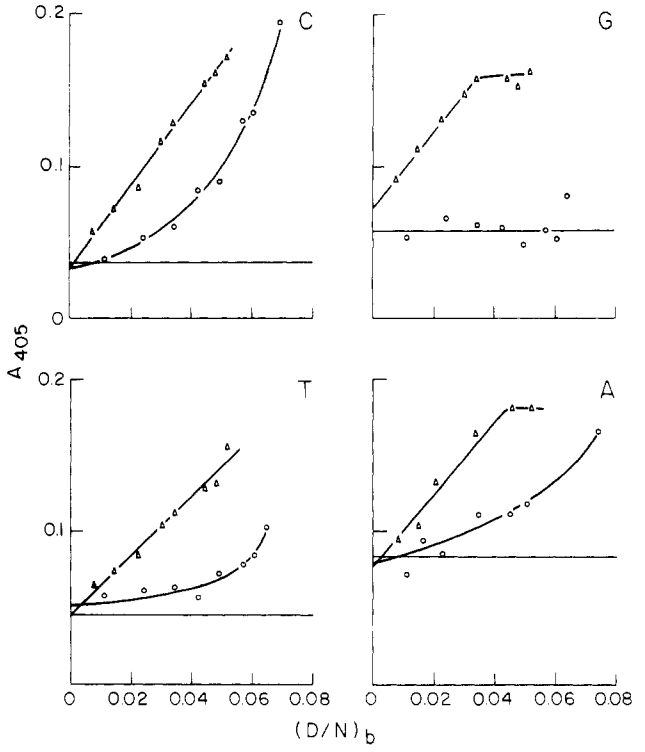

FIGURE 1: ELISA measurement of the binding of anti-cytidine (C), anti-guanosine (G), anti-thymidine (T), and anti-adenosine (A) to calf thymus DNA modified with increasing D/N of *cis*-DDP (○) and *trans*-DDP (△). Antibody binding was measured by absorbance 60 min after addition of the alkaline phosphatase substrate *p*-nitrophenyl phosphate. Background antibody binding to unmodified DNA is denoted by the solid horizontal line. Data points measured in triplicate varied on average of ∓0.007 A_{405} unit from their mean.

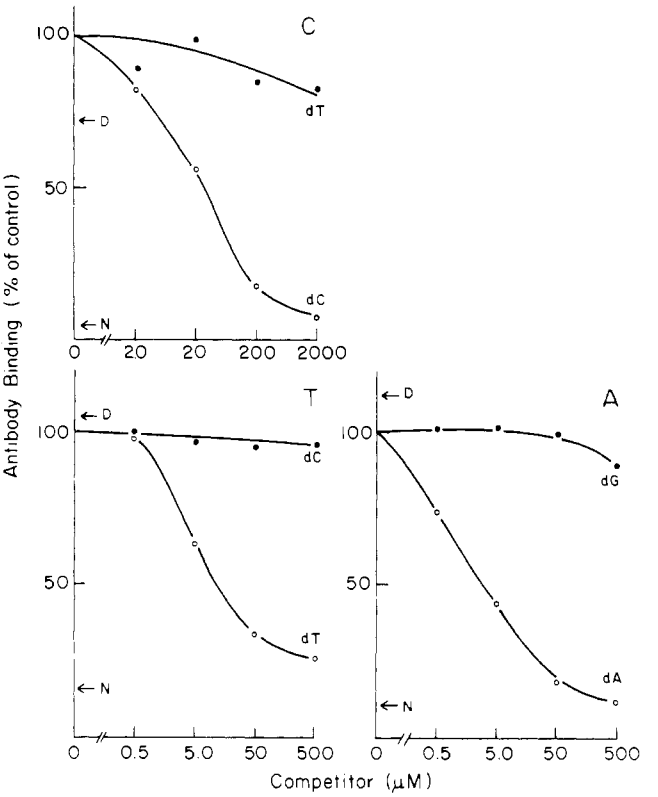

FIGURE 2: Specificity of anti-nucleoside antibody binding to *cis*-DDP–DNA (D/N = 0.12), measured by competitive immunoassay. Anti-cytidine (C), anti-thymidine (T), or anti-adenosine (A) antibody was incubated with homologous (○) or heterologous (●) nucleoside before addition to immobilized *cis*-DDP–DNA. Antibody binding measured by ELISA is reported as a percentage of binding in the absence of competitor. Levels of antibody binding to native (N) and denatured (D) DNA are denoted by arrows. Data points measured in triplicate varied an average of ∓1.8% from their mean.

detected for DNA modified with any of the platinum compounds.

Specificity of Anti-Nucleoside Antibody Binding to cis-DDP–DNA. Deoxynucleosides were used as competitors in an ELISA with the three anti-nucleoside antibodies that bind to *cis*-DDP-DNA to test whether the antibodies specifically recognized their respective nucleosides (Figure 2). Uninhibited antibody binding to highly modified, immobilized *cis*-DDP–DNA (D/N = 0.12) was comparable to binding to heat-denatured DNA and considerably higher than antibody binding to native DNA. The anti-cytidine antibody again showed the highest level of recognition, relative to denatured DNA. For each of the three antibodies, the correct deoxynucleoside inhibited antibody binding whereas other deoxynucleosides did not. Similar competitive ELISA experiments were carried out for all four anti-nucleoside antibodies with denatured DNA as the immobilized antigen (data not shown). As previously reported (Stollar, 1980) each antibody was specifically inhibited by its homologous nucleoside. Moreover, similar deoxynucleoside concentrations produced equivalent inhibition of binding to both *cis*-DDP–DNA and denatured DNA. This result indicates that the same antibody populations measure bases exposed in both denatured and *cis*-DDP-modified DNA.

Competitive Assay of Anti-Nucleoside Antibody Binding to cis-DDP–DNA. To probe further the degree to which the bases become available for antibody binding to DNA modified with relatively high D/N ratios of *cis*-DDP, binding of the four anti-nucleoside antibodies to denatured DNA was competitively inhibited with denatured DNA, native DNA, and *cis*-DDP–DNA (D/N = 0.091) (Figure 3). In each case, heat-denatured DNA strongly inhibited antibody binding while 20–50-fold higher concentrations of native DNA were required for equivalent inhibition. The activity of native DNA can be accounted for by the presence of 2–5% of denatured regions in the sample (Stollar, 1973). Modification of native DNA with *cis*-DDP, however, significantly reduced the concentration of DNA necessary for inhibition, except in the case of anti-guanosine antibodies. This finding reveals the exposure of additional regions of denaturation and is in good agreement with the measurement of direct binding to immobilized antigen.

Competitive Assay of Anti-Guanosine Antibody Binding to d(GpG)⁻ and cis-[Pt(NH₃)₂{d(GpG)}]⁺. Since guanine is known to be the favored DNA binding site of platinum complexes (Mansy et al., 1978), we investigated the possibility that coordination of *cis*-DDP at the N(7) position of guanine could block antibody recognition even when the base was not involved in Watson–Crick base pairing. As shown in Figure 4, the dinucleoside monophosphate d(GpG)⁻ competitively inhibited binding of anti-guanosine antibodies to denatured DNA whereas its *cis*-DDP adduct, *cis*-$[Pt(NH_3)_2\{d(GpG)\}]^+$, did not. This result indicates that covalent coordination of *cis*-DDP at the N(7) positions of adjacent guanines does block binding by the anti-guanosine antibodies. Deoxyadenosine was also used as a competitor to demonstrate anti-guanosine antibody specificity. Antibody binding to denatured DNA was inhibited by less than 10% in the presence of 10 μM dA.

Discussion

The anti-nucleoside antibody binding studies described here demonstrate that the bifunctional complexes *cis*- and *trans*-

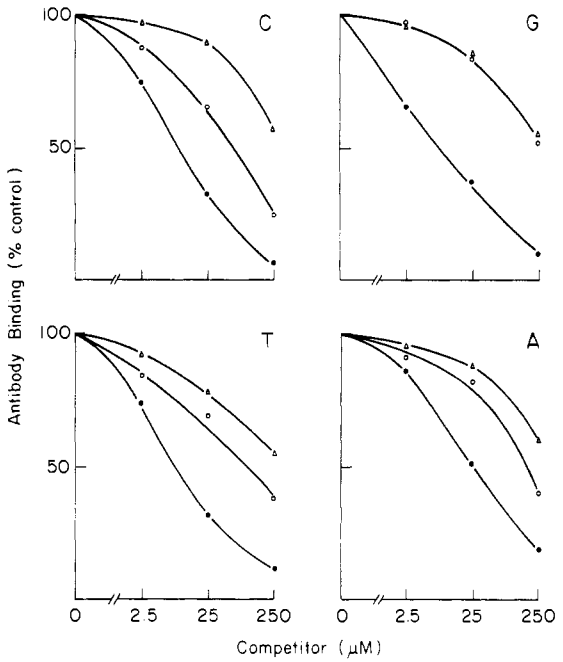

FIGURE 3: Anti-nucleoside antibody binding to heat-denatured DNA in competition with denatured DNA (●), native DNA (△), and *cis*-DDP–DNA (○) (D/N = 0.091). The concentration of competitor is reported as micromolar phosphate. Antibody binding measured by ELISA is reported as a percentage of binding in the absence of competitor. Data points measured in triplicate varied an average of ∓1.4% from their mean.

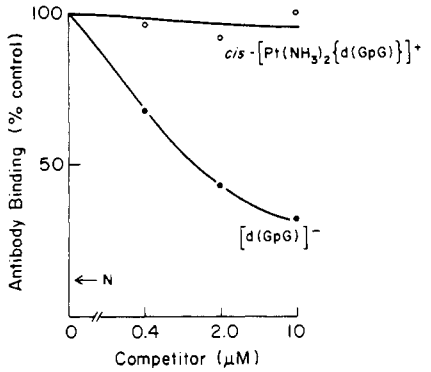

FIGURE 4: Anti-guanosine antibody binding to heat-denatured DNA in competition with $[d(GpG)]^-$ (●) and *cis*-$[Pt(NH_3)_2\{d(GpG)\}]^+$ (○). Antibody binding measured by ELISA is reported as a percentage of antibody binding in the absence of competitor. The arrow denotes the level of antibody binding to native (N) DNA. Data points measured in triplicate varied an average of ∓2.5% from their mean.

DDP alter the structure of calf thymus DNA, exposing the nucleoside bases. That this result reflects the formation of bidentate platinum adducts is strongly supported by the failure of the monofunctional $[Pt(dien)Cl]^+$ complex to expose bases in similar anti-nucleoside antibody binding experiments. There are, however, important differences in the extent to which *cis*- and *trans*-DDP adducts expose the DNA bases for anti-nucleoside antibody recognition. At a low D/N, *trans*-DDP disrupts DNA base pairing to a greater extent than does *cis*-DDP at a comparable D/N. Furthermore, modification of DNA by *trans*-DDP increases the binding of all four anti-nucleoside antibodies and is proportional to the amount of bound platinum, while antibody binding to *cis*-DDP-modified DNA depends upon the base specificity of the antibody and increases cooperatively with increasing *cis*-DDP modification levels.

These observations demonstrate that base pair disruption in a large, naturally occurring DNA accompanies the binding of *cis*- and *trans*-DDP to different degrees as measured by protein (antibody) recognition. The base specificity of this modified DNA–antibody interaction is consistent with current information about the sequence specificity of *cis*- and *trans*-DDP binding to DNA. The principal adduct formed on DNA *cis*-DDP is an intrastrand cross-link of adjacent guanines, a structure stereochemically impossible for the trans isomer. X-ray crystallography (Sherman et al., 1985), NMR spectroscopy (den Hartog et al., 1984; Van Hemelryck et al., 1984), and molecular mechanics calculations (Kozelka et al., 1985) on short oligonucleotides platinated by *cis*-DDP together indicate that such a cross-link must at least destack the adjacent guanine rings and cause substantial weakening, if not disruption, of hydrogen bonding to cytosine on the opposite strand. The present experiments allow assessment of the extent of duplex disruption in long-chain DNA where all sequences and platinum adducts exist, a choice not possible when short oligonucleotides are employed. Specifically, we find that deoxycytidine is made available for antibody binding even at low levels of *cis*-DDP modification. At higher levels of modification, where other types of *cis*-DDP–DNA adducts are known to form (Fichtinger-Schepman et al., 1985), significant binding of anti-adenosine and anti-thymidine antibodies indicates more general disruption of base pairing.

Anti-guanosine antibodies do not bind to *cis*-DDP-modified DNA, which is consistent with coordination of *cis*-DDP primarily at guanine residues since *cis*-DDP-modified $d(GpG)^-$ is not recognized by the antibodies. The failure of anti-guanosine antibodies to bind to *cis*-DDP-modified DNA at all may be rationalized as follows. At low levels of *cis*-DDP modification, mainly d(GpG) and d(ApG) adducts are formed (Fichtinger-Schepman et al., 1985). If disruption of base pairing is very localized, neither of these adducts would expose guanosine for antibody binding. Moreover, adjacent G-C base pairs, stabilized by three hydrogen bonds, are less likely to be disrupted than adjacent A-T base pairs, which are stabilized by only two hydrogen bonds. At a higher D/N of *cis*-DDP, where base pair disruption is more general, a significant percentage of the guanosine residues (21% of the bases in calf thymus DNA are guanines) is modified by *cis*-DDP and thus unable to bind to the antibodies.

At low levels of *trans*-DDP bound to DNA, replication mapping experiments indicate that platinum binding at the sequence d(GNG) (where N = dG, dA, dT, or dC) is especially favored (Pinto & Lippard, 1985). CPK space filling models show that coordination of *trans*-DDP to the N(7) position of two guanosines on the same strand, with one or more intervening nucleotides, cannot occur without breaking the interstrand hydrogen bonding of the intervening bases and causing a major disruption of local DNA structure. This picture is consistent with the observed increase in binding of all four anti-nucleoside antibodies to DNA, even at very low levels of *trans*-DDP modification. Such a major disruption of DNA structure caused by *trans*-DDP binding is consistent with recent evidence from mammalian model systems that, although *cis*- and *trans*-DDP are equally effective in blocking DNA replication, lesions made by the trans isomer are recognized and repaired much more efficiently in the cell (Ciccarelli et al., 1985). It may be that a more subtle structural perturbation induced by the cis isomer, while able to inhibit DNA replication, is less well recognized by cellular repair systems.

In summary, we observe an increase in anti-nucleoside antibody binding to DNA modified by *cis*- and *trans*-DDP but not $[Pt(dien)Cl]^+$. These results indicate that the two bi-

functional platinum complexes disrupt the normal DNA base pairing and demonstrate that protein (antibody)–DNA interactions may be altered by changes in DNA structure induced by these platinum compounds. The greater disruption observed for low levels of bound *trans*-DDP vs. *cis*-DDP correlates with the greater in vivo repair rate of the former, supporting our current hypothesis that the molecular mechanism of *cis*-DDP involves cytotoxic inhibition of DNA replication prior to recognition and repair in the cancer cell.

References

Caradonna, J. P., Lippard, S. J., Gait, M. J., & Singh, M. (1982) *J. Am. Chem. Soc. 104*, 5793–5795.

Ciccarelli, R. B., Solomon, M. J., Varshavsky, A., & Lippard, S. J. (1985) *Biochemistry 24*, 7533–7540.

Cohen, G. L., Bauer, W. R., Barton, J. K., & Lippard, S. J. (1979) *Science (Washington, D.C.) 203*, 1014–1016.

Cohen, G. L., Ledner, J. A., Bauer, W. R., Ushay, H. M., Caravana, C., & Lippard, S. J. (1980) *J. Am. Chem. Soc. 102*, 2487–2488.

den Hartog, J. H. J., Altona, C., van Boom, J. H., van der Marel, G. A., Haasnoot, C. A. G., & Reedijk, J. (1984) *J. Am. Chem. Soc. 106*, 1528–1530.

Erlanger, B. F., & Beiser, S. M. (1964) *Proc. Natl. Acad. Sci. U.S.A. 52*, 68–74.

Fichtinger-Schepman, A. M. J., van der Veer, J. L., den Hartog, J. H. J., Lohman, P. H. M., & Reedijk, J. (1985) *Biochemistry 24*, 707–713.

Girault, J.-P., Chottard, G., Lallemand, J.-Y., & Chottard, J.-C. (1982) *Biochemistry 21*, 1352–1356.

Hacker, M. P., Douple, E. B., & Krakoff, I. H., Eds. (1984) *Platinum Coordination Complexes in Cancer Chemotherapy*, Martinus Nijhoff, Boston.

Houssier, C., Macquet, M. N., & Fredericq, E. (1983) *Biochim. Biophys. Acta 739*, 312–316.

Hughes, W. L., Christine, M., & Stollar, B. D. (1973) *Anal. Biochem. 55*, 468–478.

Inagaki, K., & Kidani, Y. (1980) *Inorg. Chim. Acta 46*, 35–39.

Kanai, Y., Tauchi, M., Aotsuka, S.-I., & Yokohari, R. (1982) *J. Immunol. Methods 53*, 355–365.

Kozelka, J., Lippard, S. J., Quigley, G., & Petsko, G. A. (1985) *J. Am. Chem. Soc. 107*, 4079–4081.

Lippard, S. J., Ushay, H. M., Merkel, C. M., & Poirier, M. C. (1983) *Biochemistry 22*, 5165–5168.

Macquet, J.-P., & Butour, J.-L. (1978) *Biochimie 60*, 901–914.

Mansy, S., Chu, G. Y. H., Duncan, R. E., & Tobias, R. S. (1978) *J. Am. Chem. Soc. 100*, 607–616.

Pinto, A. L., & Lippard, S. J. (1985) *Proc. Natl. Acad. Sci. U.S.A., 82*, 4616–4619.

Poirier, M. C. (1981) *JNCI, J. Natl. Cancer Inst. 67*, 515–519.

Poirier, M. C., Lippard, S. J., Zwelling, L. A., Ushay, H. M., Kerrigan, D., Thill, C. C., Santella, R. M., Grunberger, D., & Yuspa, S. H. (1982) *Proc. Natl. Acad. Sci. U.S.A. 79*, 6443–6447.

Polissiou, M., Viet, M. T. P., St-Jacques, M., & Theophanides, T. (1985) *Inorg. Chim. Acta 107*, 203–210.

Roberts, J. J., & Pera, M. P. (1983) in *Molecular Aspects of Anti-Cancer Drug Action* (Neidle, S., & Waring, M. J., Eds.) pp 183–231, Macmillian Press, London.

Royer-Pokora, B., Gordon, L. K., & Haseltine, W. A. (1981) *Nucleic Acids Res. 9*, 4595–4609.

Sandberg, A. L., & Stollar, B. D. (1966) *J. Immunol. 96*, 764–771.

Schreck, R. R., Erlanger, B. F., & Miller, O. J. (1974) *Exp. Cell Res. 88*, 31–39.

Scovell, W. M., & Capponi, V. J. (1984) *Biochem. Biophys. Res. Commun. 124*, 367–374.

Seaman, E., Levine, L., & Van Vunakis, H. (1968a) in *Nucleic Acids in Immunology* (Plesicia, O. J., & Braun, W., Eds.) pp 157–164, Springer-Verlag, New York.

Seaman, E., Levine, L., & Van Vunakis, H. (1968b) in *Nucleic Acids in Immunology* (Plesicia, O. J., & Braun, W., Eds.) pp 165–173, Springer-Verlag, New York.

Sherman, S. E., Gibson, D., Wang, A. H.-J., & Lippard, S. J. (1985) *Science (Washington, D.C.) 230*, 412–417.

Sproat, B. S., & Gait, M. J. (1984) in *Oligonucleotide Synthesis; A Practical Approach* (Gait, M. J., Ed.) pp 83–115, IRL Press, Oxford.

Stollar, B. D. (1973) *Antigens 1*, 1–85.

Stollar, B. D. (1980) *Methods Enzymol. 70*, 70–85.

Stone, P. J., Kelman, A. D., & Sinex, F. M. (1974) *Nature (London) 251*, 736–737.

Strickland, P. T., & Boyle, J. M. (1984) *Prog. Nucleic Acid. Res. Mol. Biol. 31*, 1–58.

Tullius, T. D., & Lippard, S. J. (1981) *J. Am. Chem. Soc. 103*, 4620–4622.

Ushay, H. M., Tullius, T. D., & Lippard, S. J. (1981) *Biochemistry 20*, 3744–3748.

Van Hemelryck, B., Guittet, E., Chottard, G., Girault, J.-P., Huynh-Dinh, T., Lallemand, J.-Y., Igolen, J., & Chottard, J.-C. (1984) *J. Am. Chem. Soc. 106*, 3037–3039.