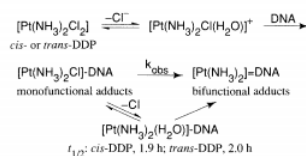


9.3: Aspects of Platinum Binding to DNA

Given that DNA is a major target of platinum binding in cells, it is incumbent upon the bioinorganic chemist to investigate the nature of these interactions and their biological consequences. Of all the ligands studied in coordination chemistry, DNA is surely among the most complex. In the ensuing discussion, we first present experiments that delineate the chemical steps involved in *cis*- and *trans*-DDP binding to DNA as well as the chemical consequences of the adducts formed. We next describe the physical changes in the double helix that accompany platinum binding, and then we discuss the biological consequences that attend the platination of DNA. Subsequent sections describe the major adducts formed, in other words the regiospecificity of the drug, the three-dimensional structures of the adducts, and the way in which different structures within DNA can modulate platinum binding. Finally, we consider the response of the cell to Pt-DNA adducts, including studies with site-specifically modified DNA, and speculate about how this chemistry might relate to the antitumor drug mechanism.

a. Kinetics of Platinum Binding to DNA

The binding of *cis*- and *trans*-DDP to DNA has been studied⁶⁷ by ¹⁹⁵Pt NMR spectroscopy with the use of isotopically enriched ¹⁹⁵Pt, which has a nuclear spin $I = \frac{1}{2}$. The DNA used in this experiment was obtained from chicken red blood cell chromosomes that had been enzymatically degraded to relatively small pieces ranging from 20 to 60 base pairs in length (molecular-weight range 13 to 30 kDa). Since the ¹⁹⁵Pt chemical shifts are very sensitive to chemical environment, this NMR study provided important details about the kinetics and mechanism of platinum binding to the biopolymer. The rate-determining step in platination of the DNA is loss of chloride ion (Equation 9.5) to form the mono-aqua complex, which rapidly coordinates to a nitrogen donor on the nucleic acid. The identification of the coordinating atom as nitrogen was possible because the ¹⁹⁵Pt chemical shift is characteristic of species having one chloride and three nitrogen ligands bound to Pt(II).⁶⁷ The spectroscopic changes that accompany the formation of the family of monofunctional adducts are shown in Figure 9.8. Subsequent hydrolysis of the second chloride ion leads to the formation of a second bond with DNA. This sequence of events affords bifunctional adducts and is similarly accompanied by discrete ¹⁹⁵Pt spectral changes (Figure 9.8). From the ¹⁹⁵Pt chemical-shift range of the final products, it was apparent that the *cis*-{Pt(NH₃)₂}²⁺ moiety is bound primarily to two nitrogen donors on the nucleic acid. This chemistry is summarized in Equation (9.10), together with the half-lives for the mono-functional adducts. The half-lives were calculated from a kinetic analysis of the time-dependence of the ¹⁹⁵Pt spectral changes. As can be seen, the rates of closure of mono- to bifunctional adducts for the two isomers are quite similar, suggesting that their different biological properties are not a consequence of the kinetics of binding to DNA.



(9.10)

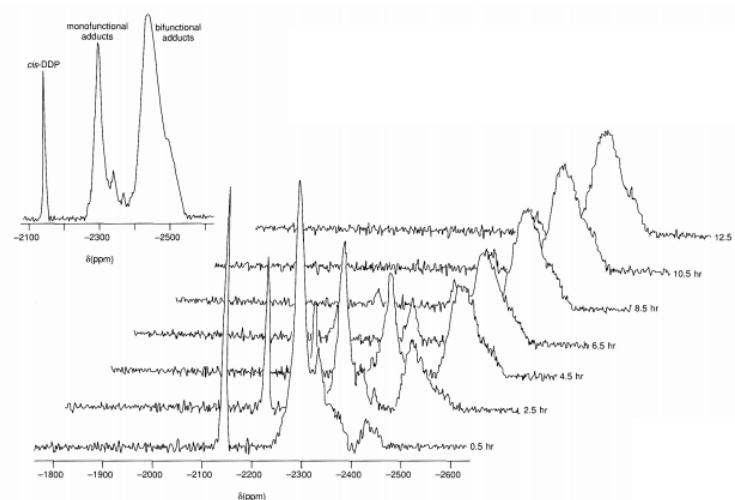


Figure 9.8 - Time course of the reaction between double-stranded chicken erythrocyte DNA and $\text{cis-}[\text{Pt}(\text{NH}_3)_2(\text{H}_2\text{O})\text{Cl}]^+$ at a D/N = 0.07, in 3 mM NaCl and 1 mM NaH_2PO_4 , 37 °C, pH 6.5. Each spectrum consists of 200,000 transients. The inset shows the sum of the individual spectra (reproduced by permission from Reference 67).

The next logical question to address is what donor atoms on DNA are coordinating to platinum in the mono- and bifunctional adducts. This important issue is discussed in considerable detail in Sections V.D.4 and V.D.5. As will be shown, the N7 atoms of the purine bases adenine and guanine are the principal binding sites. Alkylation of DNA at these positions facilitates depurination. Platinum binding to N7 atoms of purines (Figure 9.9), however, stabilizes the glycosidic (N9-C1') linkage.⁶⁸⁻⁷⁰ Presumably the positive charge is better distributed over the platinum atom and its ligands in the adduct than over a purine alkylated at N7. On the other hand, platinum binding to N7 of guanine does perturb the charge distribution in the purine ring, as evidenced by the lowering of the pK_a of N1 by ≈ 2 units from its value in the unplatinated nucleotide (usually from $\text{pK}_a \approx 10$ to $\text{pK}_a \approx 8$).^{71,72} This effect has been used to assign platinum binding sites in DNA fragments, as discussed below.

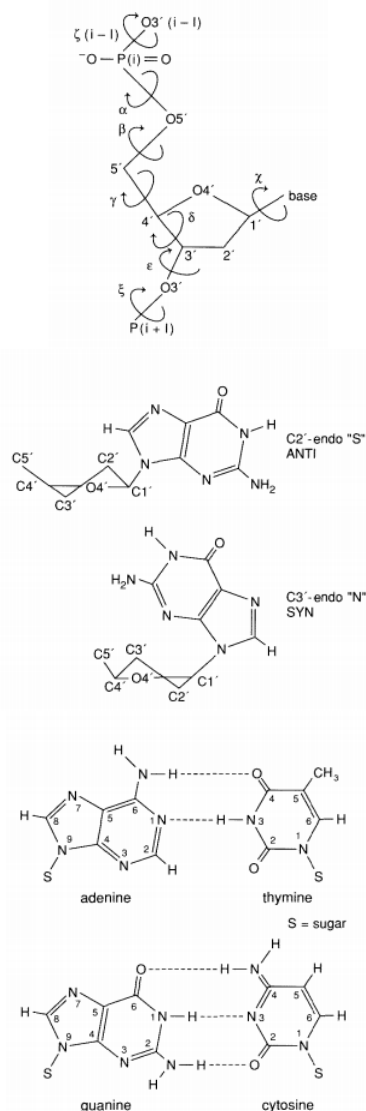


Figure 9.9 - Structural components of DNA. The left panel shows a schematic of the DNA backbone, including the deoxyribose ring numbering scheme and torsion angles. The center panel gives the two main classes of sugar pucker and base orientations. The right panel gives the bases in their Watson-Crick, A-T, and G-C pairs and the base numbering scheme, viewed down the helix axis, with the major grooves pointing toward the top of the figure and the minor grooves toward the bottom.

What are the chemical changes at the platinum center when *cis*-DDP binds to DNA? Both chloride ions are lost from the coordination sphere, as already indicated. Platinum EXAFS studies of calf-thymus DNA modified with *cis*-DDP revealed no chlorine backscattering features characteristic of Pt-Cl bonds.⁷³ The spectra were consistent with the presence of four Pt-N/O linkages, since the technique is unable to distinguish between the two low-Z elements oxygen and nitrogen. Various studies reveal that, under most circumstances, the NH₃ ligands are not lost from DNA upon the binding of platinum ammine halides. For example, when ¹⁴C-labeled *cis*-[Pt(NH₂CH₃)₂Cl₂] was allowed to bind to T7 (47 percent GC content) or *M. luteus* (73 percent GC content) DNA, no loss of radiolabel was found to accompany platinum binding.⁷⁴ *In vivo*, however, loss of amine ligands has been observed. Injection of ^{195m}Pt and ¹⁴C doubly labeled [Pt(en)Cl₂] into tumor-bearing mice resulted in unequal distribution of the two labels in various biochemical fractions, but there is no reason to believe that this result is relevant to the antitumor mechanism.⁷⁵ Metabolic inactivation of the drug could occur in a variety of ways unrelated to anticancer activity. The best evidence that ammine loss does not occur at the critical biological target of cisplatin is the finding, by using antibodies specific for *cis*-{Pt(NH₃)₂}²⁺ nucleotide complexes (see Section V.D.4.c), that DNA, extracted from cells in culture or from human cancer patients treated with *cis*-DDP and subsequently degraded, contains intact Pt-NH₃ linkages.⁷⁶

Once bonds are made between Pt and its targets on DNA, they are relatively inert kinetically. Platinum-DNA complexes can be subjected to various physical methods of separation and purification, including gel electrophoresis, ethanol precipitation,

centrifugation, and chromatography, as well as to enzymatic and even chemical degradation procedures that digest the DNA, without releasing the platinum. Platinum can be removed, however, either by use of cyanide ion, to form the very stable ($K \sim 10^{41}$) $[\text{Pt}(\text{CN})_4]^{2-}$ complex, or by excess thiourea.^{77,78} These properties have proved to be extremely valuable in facilitating localization and characterization of the major *cis*- and *trans*-DDP binding sites on DNA.

Although Pt-DNA linkages are, generally speaking, kinetically inert, sometimes a particular adduct will rearrange into a more stable linkage isomer. One interesting example is the product of the reaction of *trans*-DDP with the dodecanucleotide 5'-d(TCTACGCGTTCT).⁷⁹ Initially the platinum coordinates to the two guanosine residues, forming a *trans*- $[\text{Pt}(\text{NH}_3)_2\{\text{d}(\text{GCG})\}]$ 1,3-intrastrand crosslink. This complex rearranges to a more stable *trans*- $[\text{Pt}(\text{NH}_3)_2\{\text{d}(\text{CGCG})\}]$ 1,4-intrastrand crosslink with a half-life of 129 h at 30 °C or 3.6 h at 62 °C. In this rearrangement product the platinum is coordinated to a cytosine and a guanosine residue.

As just described, the binding of bifunctional platinum complexes to DNA proceeds in a stepwise fashion. The second step is sufficiently slow (a few hours), however, that various reagents such as NH_3 , nucleobases, and low concentrations of thiourea can coordinate to the fourth site and trap the monoadducts. Generally speaking, however, given sufficient time both *cis*- and *trans*-DDP will bind DNA in a bifunctional manner. As such, they bear some resemblance to organic alkylating agents, such as the nitrogen mustards, which have been employed as anticancer agents.⁸⁰

b. Crosslinking Reactions of Platinum Complexes

There are three broad classes of DNA adducts that can be made by bifunctional platinum complexes. As illustrated for *cis*-DDP in Figure 9.10, they are DNA-protein crosslinks, interstrand DNA-DNA crosslinks, and intrastrand crosslinks.⁸¹ A fourth possibility for platinum complexes is bidentate chelate ring formation utilizing two donor atoms on a nucleotide. For many years, a favored such postulated mode of binding was chelation by the N7-O6 positions of the guanine base (Figure 9.9), since this structure could be formed only by *cis*- and not by *trans*-DDP.^{82,83}

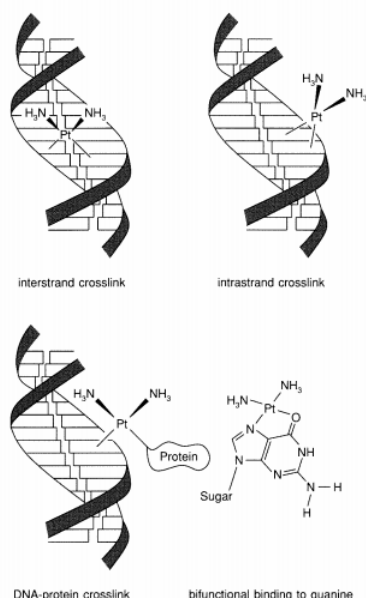


Figure 9.10 - Possible bifunctional binding modes of *cis*-DDP with DNA (reproduced by permission from Reference 81).

Such a structure has never been observed for *cis*-DDP binding to DNA, however. DNA-protein and interstrand crosslinks formed by platinum complexes have been the focus of many attempts to explain cytotoxicity and antitumor behavior.⁸⁴ The technique of alkaline elution, in which crosslinked DNA-DNA strands or DNA-protein molecules bind to filter paper following denaturation under basic conditions, sensitively and easily reveals such adducts. *trans*-DDP forms such adducts more rapidly than the *cis* isomer, perhaps because of its faster chloride-ion hydrolysis rates (see above) and a more favorable geometry, but they also seem to be repaired more rapidly in cells. As will be shown, interstrand and DNA-protein crosslinks are a small minority of adducts formed by cisplatin, and their contribution to the cytotoxic and anticancer properties of the drug remains to be established. In studies of SV40 replication *in vivo*, DNA-protein crosslinking by *cis*- and *trans*-DDP was shown not to be correlated with the inhibition of DNA replication.⁸⁵

What proteins form crosslinks to DNA? One possibility is the histones that make up the spools around which DNA is wound when packaged into chromatin in the nucleus. Studies⁸⁶ of *cis*- and *trans*-DDP binding to nucleosome core particles (each particle made up of eight histone proteins; around each particle is wound a 146-bp piece of DNA in a shallow superhelix of 1.75 turns) revealed the DNA binding to be little affected by the protein core. Both DNA-protein and specific histone crosslinked species were observed; from the latter it was suggested that DDP complexes might be useful crosslinking probes of biological structures. Other proteins likely to form crosslinks to DNA in the presence of platinum complexes are DNA-processing enzymes, or enzymes requiring a DNA template for normal function. In the *in vivo* SV40 study, for example, T antigen was one of the proteins found to be crosslinked to SV40 DNA by cisplatin.⁸⁵ Other nuclear proteins such as the high-mobility group (HMG) class are also crosslinked to DNA in the presence of *cis*-DDP. In all cases so far, DNA-protein crosslinking has occurred when platinum was added to cells. There is as yet no evidence that transfection of platinated DNA into cells results in such crosslinking or that crosslinks form during *in vitro* enzymatic digestions of platinated DNAs.

c. Physical Effects of Platinum-DNA Binding

(i). Unwinding, Shortening, and Bending of the Double Helix

Early studies of *cis*- and *trans*-DDP binding to DNA employed closed and nicked circular plasmids.⁸⁷ As was described in more detail in Chapter 8, closed circular DNAs are topologically constrained such that any change in the number of helical turns must result in an equal and opposite number of superhelical turns. Any reagent that unwinds the double helix reduces the number of helical turns. Consider, for example, a stretch of DNA that is 360 base pairs (bp) long. Normal B-DNA has ≈ 10.5 bp per turn or a helical winding angle of $\approx 34.3^\circ$ per bp. Suppose the DNA is unwound, so that there are now 12 bp per turn or a winding angle of $\approx 30^\circ$. Instead of 34.3 helical turns ($360 \div 10.5$), the DNA now has only 30 ($360 \div 12$). If this DNA molecule were in the form of a covalently closed circle, the helical unwinding of -4.3 turns would be accompanied by a superhelical winding of +4.3 turns.

Planar organic dyes such as ethidium bromide (EtdBr) and inorganic complexes such as $[\text{Pt}(\text{terpy})(\text{HET})]^+$ (Figure 9.11) bind to DNA by intercalation, inserting between the base pairs and unwinding the double helix by $\sim 26^\circ$ per molecule bound (Figure 9.12).⁸⁸ This unwinding can be measured by monitoring changes in the superhelicity of closed circular DNA. This kind of DNA is subjected to certain topological constraints that lead to the formation of supercoils and superhelical winding that dramatically alter the hydrodynamic properties of the DNA. Either gel electrophoresis or analytical ultracentrifugation can be used to measure this phenomenon. The platinum complexes *cis*- and *trans*-DDP also produce changes in the superhelix density when bound to closed circular DNA.⁸⁷ As shown in Figure 9.13, increasing concentrations of platinum bound per nucleotide on the DNA first retard its mobility and then increase its mobility through the gel. These interesting alterations in gel mobility occur because the negatively coiled superhelix unwinds first into an open, or untwisted, form and then into a positively supercoiled form. The conformational changes, which are depicted in Figure 9.14, are directly proportional to the drug-per-nucleotide, or $(D/N)_b$, ratio. In addition to superhelical winding, both platinum complexes increase the mobility of nicked circular DNA in the gels (Figure 9.13). Nicked DNA has one or more breaks in the sugar-phosphate backbone, which relieve the topological constraint and prohibit the DNA from twisting into superhelical structures.

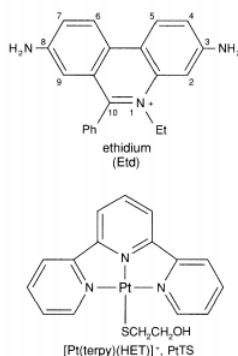


Figure 9.11 - Organic (top) and inorganic (bottom) intercalators.

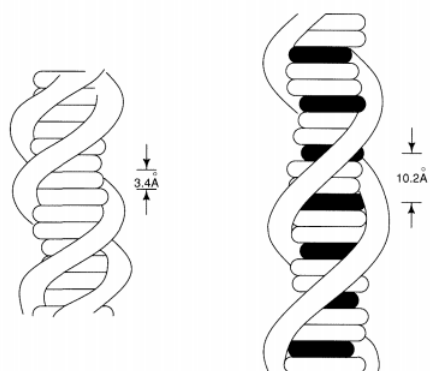


Figure 9.12 - Schematic representation of double-stranded DNA without (left) and with (shaded area, right) a bound intercalator (reproduced with permission from Reference 51).

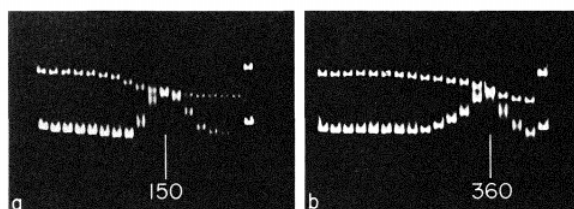


Figure 9.13 - Electrophoresis in 1 percent agarose gels of nicked and closed circular PSMI DNA incubated with (a) *cis*- and (b) *trans*-[Pt(NH₃)₂Cl₂] as function of time. After electrophoresis, gels were stained with ethidium bromide. Reproduced with permission from Reference 51.

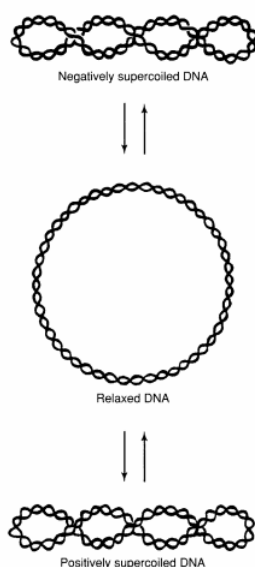


Figure 9.14 - Topological forms of closed circular DNA.

What could be the cause of these physical changes in the DNA structure upon *cis*- or *trans*-DDP binding? Intercalation can be excluded, not only because the compounds do not have the aromatic character normally associated with intercalators (Figure 9.11), but also through studies of the manner by which these and other platinum complexes inhibit the intercalative binding of EtdBr to DNA.^{89,90} Platinum metallointercalators such as [Pt(terpy)(HET)]⁺ are competitive inhibitors of EtdBr binding, as measured by fluorescence Scatchard plots, whereas the non-intercalators *cis*- and *trans*-DDP are not. Moreover, intercalation tends to lengthen and stiffen the double helix, whereas the mobility changes of nicked circular DNAs upon binding of *cis*- or *trans*-DDP were shown by electron microscopy experiments to arise from a pronounced shortening of the DNA with increased Pt binding.

One manner by which *cis*- or *trans*-DDP might produce these physical alterations in DNA structure is by kinking the double helix at or near the binding site. Such an effect could be produced by the bidentate attachment of platinum; the monofunctional [Pt(dien)Cl]⁺ complex does not have these pronounced effects on DNA secondary structure.⁹¹ Recently, it has been demonstrated

that *cis*-DDP binding to DNA does indeed produce a pronounced bend in the helix axis.^{92,93} The proof employed a gel-electrophoretic method of analysis that had previously been used to study DNA bending at naturally occurring specific sequences called A-tracts, consisting of five or six adenosine nucleosides in a row followed by about the same number of thymidine residues.⁹⁴ When these d(A₅T₅)₂ sequences are positioned in the center of a DNA restriction fragment of, say, 150 bp, the mobility of the DNA through polyacrylamide electrophoresis gels is greatly retarded compared to that of a similar DNA fragment where the A-tract is at the end. For the former fragment, the bent molecules presumably cannot snake their way through the pores of the polyacrylamide as well as the molecules whose bends are at the ends and have little effect on the linear structure. It was further shown that A-tracts bend the duplex toward the minor groove of the DNA. Moreover, in a DNA containing multiple A-tracts, the bends must be separated by integral numbers of helical turns (~ 10.5 bp) or else the effect will cancel and the gel mobility will be that of normal DNA of similar length. This latter phenomenon has been referred to as phasing.

With this background information in mind, we can now discuss the experiments with *cis*-DDP that demonstrated bending.^{92,93} By methods described in Section V.D.8, a 22-bp oligonucleotide (22-mer) containing self-complementary overhanging ends ("sticky ends") was synthesized with a single *cis*-diammineplatinum(II) moiety linking adjacent guanosine residues (Figure 9.15A). A 22-mer was chosen since it has approximately two helical turns, accounting for some platinum-induced unwinding, and will thus have phased bends when polymerized. This platinated DNA was then labeled with ³²P and treated with the enzyme DNA ligase, which seals the ends, producing oligomers of the 22-mer having lengths 22, 44, 66, 88, 110, etc., bp. In these oligomers, the platinum atoms are spaced apart approximately by integral numbers of helical turns. As shown in Figure 9.15, studies of this family of oligomers by gel electrophoresis revealed a pronounced retardation compared to the mobility of unplatinated DNA oligomers of comparable size (line P22 in Figure 9.15B). The plots in this figure show the relative mobilities (R_L) of the different length multimers, compared to a control in which the top strand is not platinated, as a function of the length in base pairs. From the resulting curves may be extracted the extent of cooperative bending. When oligomers of a platinated DNA fragment in which the metal atoms were spaced apart by 27 bp were examined, their relative mobilities were found to be nearly the same as unplatinated control molecules (line P27 in Figure 9.15B). These experiments unequivocally established that platinum kinks the double helix. As with A-tract-induced bends, the platinum atoms must be phased in order to induce cooperative bending. Comparison of the magnitudes of the gel mobility changes made it evident that *cis*-{Pt(NH₃)₂}²⁺ binding produces a bend comparable to that of two A-tracts, ~ 34°.

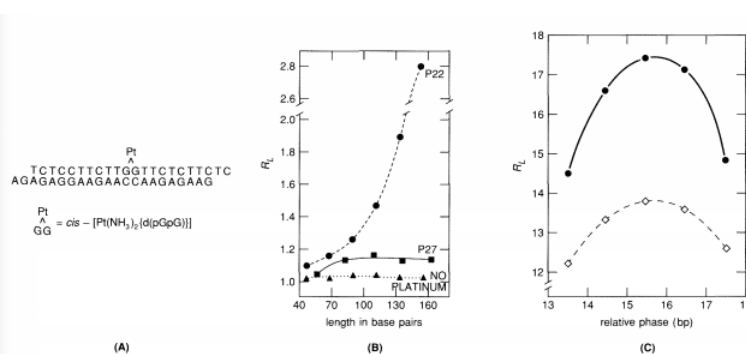


Figure 9.15 - Experiment to demonstrate that *cis*-[Pt(NH₃)₂{d(pGpG)}] intrastrand crosslinks bend duplex DNA by ~ 34°. Panel A shows the platinated 22-mer sequence, panel B the effect of platination on the gel-electrophoresis mobility of the 22-mer (P22) and a control 27-mer (P27) oligomers, and panel C the mobility of copolymerized DNAs containing *cis*-DDP and A-tract induced bends (●), 128-bp Pt + A-tract DNA; ◇, 96-bp Pt + A-tract DNA) that maximize at approximately halfintegral helical turns corresponding to their phasing. For more detail consult Reference 93.

In a related series of experiments,⁹³ the platinated 22-mer was copolymerized with various A-tract-containing 12-mers to produce ladders of oligomers in which the phasing of Pt with respect to the center of the A-tract was varied, but the Pt atoms were always in phase. The results of these studies showed that maximum gel-mobility retardation occurred when the Pt and A-tract center were spaced apart by half-integral numbers of helical turns (Figure 9.15C). Since A-tracts bend the DNA into the minor groove, this result implies that platinum bends the DNA into the major groove. Only when phased by $n/2$ (n = integer) turns will copolymers of species situated alternatively in the major and minor grooves of DNA exhibit such cooperative bending. It will be shown later that helix bending of *cis*-DDP-DNA adducts into the major groove is in accord with their known structures.

The ability to prepare site-specifically platinated oligonucleotides (see Section V.D.8) has provided a means for measuring the extent to which *cis*-DDP produces local unwinding of the double helix.⁹⁵ When the platinum atoms are positioned with respect to one another, or phased, by exactly integral numbers of helical turns, the retardation of the DNA multimers in the gel is maximized.

This phenomenon is illustrated in Figure 9.16, where the R_L values are plotted as a function of the interplatinum spacing for oligonucleotides containing the cis - $\{Pt(NH_3)_2d(GpG)\}$ intrastrand crosslink. When the resulting curve was analyzed, the maximum was found to occur at 21.38 bp. Since normal B-DNA has a helical repeat of 10.5 bp, one can compute the effect of platination from the expression $[(21.38 - 2(10.5))] \text{ bp} = 0.38 \text{ bp}$. From the fact that one helical turn of DNA comprises 360° and 10.5 bp, the unwinding of the DNA double helix due to the presence of a single cis - $\{Pt(NH_3)_2d(GpG)\}$ intrastrand crosslink can be calculated as

$$\left(\frac{0.38}{10.5}\right) \times 360 = 13^\circ. \quad (9.3.1)$$

Similar studies of DNA platinated with *trans*-DDP have been carried out. In these, oligonucleotides containing the 1,3-*trans*- $\{Pt(NH_3)_2d(GpNpG)\}$ intrastrand crosslink were examined. The electrophoresis gels of polymerized 15-mers and 22-mers containing this adduct showed cooperative bending. This result indicates that bends at the sites of platination by *trans*-DDP are not phase sensitive, and has been interpreted to imply the formation of a "hinge joint" at these positions.^{92,95} The directed bends and local unwinding of DNA produced by cisplatin could be an important structural element that triggers a response by cellular proteins. This subject is discussed in greater detail in Section V.D.7.d.

d. Biological Consequences of Platinum-DNA Binding

(i). Inhibition of Replication

Binding of *cis*-DDP to DNA inhibits replication both *in vivo* and *in vitro*, as shown by a variety of assays. Inhibition of replication of SV40 viral DNA in African green monkey cells as a function of the concentration of added *cis*-DDP is shown in Figure 9.17. When SV40 virus infects monkey cells, it does not integrate its DNA into the genome of the host. Instead, it forms its own chromosomes in the cell nucleus. These so-called mini-chromosomes consist of ≈ 20 nucleosomes, fundamental chromosome building blocks. SV40 has its own life cycle, using virally encoded and cellular proteins to replicate and, ultimately, reassemble virus particles before lysing the cell and departing to infect neighboring cells.

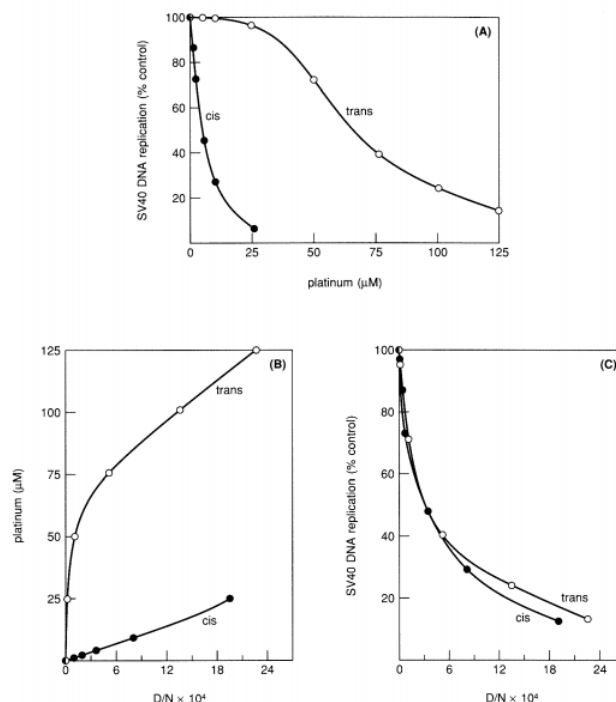


Figure 9.17 - SV40 DNA replication in CV-1 cells as a function of platinum concentration in the medium (panel A) or D/N (panel C). In panel B, DIN is plotted as a function of platinum concentration in the medium. SV40-infected cells were treated with *cis*-DDP (●) or *trans*-DDP (○) at the indicated concentrations for 40 h. SV40 DNA replication relative to control (untreated) cells was measured by incorporation of $[^3H]$ thymidine, added after the first 24 h of platinum treatment, and Pt in isolated SV40 chromosomes was measured by AAS. The data shown are from a representative experiment. Experiments were carried out in quadruplicate. Reproduced by permission from Reference 85.

In the experiment shown in Figure 9.17, the SV40-infected cells were treated with cisplatin. After 24 h, [^3H]thymidine was added and, after 24 more hours, the cells were harvested, and SV40 DNA was isolated; the amount of DNA synthesis was recorded by comparing incorporated radiolabel with results from control experiments where no platinum was present. The data show that, when 25 μM platinum was present, SV40 DNA replication was reduced to about 5 percent of control. Quantitation reveals that, at ≈ 2 platinum atoms bound per thousand nucleotides (drug-per-nucleotide, or $(\text{D/N})_{\text{b}} = 0.002$), synthesis is only 10 percent that of control.

Recently, a related series of experiments has been carried out that can monitor DNA synthesis from templates platinated *in vitro*.⁹⁶ In this work, DNA plasmids containing the SV40 origin of replication are added to cellular extracts prepared from human kidney cells previously infected with adenovirus. In the presence of large T antigen, a virally encoded protein required for replication, SV40 DNA is synthesized from the plasmid templates. Synthesis can be conveniently monitored by [^{32}P]dATP incorporation. At a $(\text{D/N})_{\text{b}}$ ratio of only 1.7×10^{-3} , DNA synthesis is about 5 percent of control, in agreement with the results of the *in vivo* study.

The binding of *cis*-DDP to DNA has also been measured for normal and tumor cells implanted in nude mice and in cells obtained from the ascites fluid of patients with ovarian carcinoma 24 h after their last dose.⁹⁷ The data for mouse bone marrow and a human pancreatic tumor xenograft show that, at a dose of 10 mg/kg, (D/Nh) platinum binding levels of 3.3×10^{-6} and 1.82×10^{-6} reduce survival to 20 and 10 percent of control, respectively. These ratios are in good accord with platinum levels required to inhibit DNA synthesis in mammalian cells, as revealed by various studies, but substantially less than that needed for replication inhibition in the SV40 experiments described above. The difference can be readily explained, illustrating an important point. The SV40 genome, like most other DNAs of viral or plasmid origin, consists of only 15,000 nucleotides whereas the nuclear DNA of mammals has about 10^9 nucleotides. Thus, $(\text{D/N})_{\text{b}}$ levels of $\sim 10^{-6}$ would leave 99 out of 100 SV40 DNA molecules with no platinum at all, and replication would hardly be affected. For the mammalian genome, $(\text{D/N})_{\text{b}}$ values of 10^{-6} place 10^3 platinum atoms on each DNA genome, sufficient to inhibit replication and reduce cell survival. Platinum-DNA binding levels of this magnitude are found for ovarian ascites cells taken from patients receiving cisplatin chemotherapy.⁹⁷

(ii). Mutagenesis and Repair

Apart from inhibition of DNA synthesis, what are the other biological consequences of cisplatin binding to DNA? One such consequence is mutagenesis, in which a normal base in the sequence is replaced by a different base. This phenomenon has been demonstrated for *cis*-DDP-treated cells in a variety of studies. What brings about such mutagenesis? There are several possibilities. One is that errors are introduced in DNA strands during attempts of the replication apparatus to synthesize past a platinum lesion. Another is that the platinum-damaged DNA is recognized by cellular repair systems that, in attempting to eliminate the platinated stretch of DNA, incorporate one or more incorrect nucleotides. Platinum-induced mutagenesis can lead to deleterious long-term health problems in patients treated with cisplatin. It is therefore important to understand the mechanism by which cellular DNA becomes mutated following platination, and to devise strategies for minimizing or eliminating this mutagenesis.

The foregoing considerations bring up another biological consequence of *cis*-DDP binding, namely, DNA repair. Removal of platinum from DNA by cellular repair mechanisms has been demonstrated by several groups. For example, in studying *cis*-DDP-treated human fibroblast cells in culture, it was found that the amount of bound platinum per nucleotide decreased according to first-order kinetics, from $(\text{D/N})_{\text{b}}$ of 2.3×10^{-5} to 3.3×10^{-6} over a six-day period. Since Pt-DNA adducts are stable with respect to dissociation from DNA under physiological conditions (see above discussion), loss of platinum was attributed to DNA repair.⁹⁸

How does the cell remove platinum from DNA? One mechanism is by a process known as excision repair, whereby the sugar-phosphate backbone on the platinated strand is hydrolyzed ("nicked") on either side of the damage and the remaining, unplatinated strand is used as a template for new DNA synthesis. The platinated oligonucleotide is displaced and the resulting gap filled in. In support of this picture is the fact that, in xeroderma pigmentosum (XP) human fibroblast cells, known to be deficient in excision repair, there is very little removal of platinum during post-treatment incubation.⁹⁹ Recent studies of *in vitro* repair of cisplatin-DNA adducts by a defined enzyme system, the ABC excision nuclease of *E. coli*, have provided some details at the molecular level about the process.^{100,101} [^{32}P]-Labeled double-stranded DNA fragments containing $\{\text{Pt}(\text{NH}_3)_2\}^{2+}$ or $\{\text{Pt}(\text{en})\}^{2+}$ adducts at random or defined sites were incubated with the enzyme. Cleavage of the platinated strand occurred at the 8th phosphodiester bond 5', and the 4th phosphodiester bond 3', to the GG or AG intrastrand crosslink. Further details about the identification and construction of such specific crosslinks will be given later in this chapter.

(iii). Drug Resistance

Another biological consequence of DNA-platinum interactions, probably related to the repair phenomenon, is resistance. Resistance of a cell to a chemotherapeutic agent, which can be inherent or acquired, is a phenotypical ability of the cell to tolerate

doses of a drug that would be toxic to normal, or parent, cells.¹⁰² Resistance is often acquired by prolonged exposure of cells in culture to the drug or, in patients, to repeated doses of drug therapy. There is not yet any direct proof that platinum-DNA interactions are responsible for acquired resistance to cisplatin. Studies of sensitive and resistant tumors in rats have shown, however, that after intravenous injection of 10 mg/kg of the drug, the platinum levels were the same after an hour, but after 24 hours a larger proportion of adducts had been removed in the resistant cells.¹⁰³ Similar results have been found for studies of Pt-DNA adducts in cultured L1210 cells of varying levels of resistance to cisplatin where, in the 18 h following a 6-hour incubation with the drug, the resistant cells had up to fourfold more platinum removed than the sensitive cells.¹⁰⁴

Experiments have also been carried out showing that *cis*-DDP binding to DNA inhibits transcription, the formation of RNA from a gene, and that this phenomenon is less efficiently reversed for parent versus resistant L1210 cells in culture.¹⁰⁵ The assay involves transfection (the process whereby free or viral DNA or RNA is taken into a cell) of pRSVcat plasmid DNA into L1210 cells. The plasmid contains the bacterial *cat* gene in a position that permits its expression in mammalian cells. The *cat* gene encodes the enzyme chloroamphenicol acyltransferase (CAT), an activity readily measured following lysis of the cells. Transfection of the *cis*-DDP-damaged plasmid into resistant L1210 cells showed that up to eight times the amount of platinum was required in the resistant versus sensitive cells to produce a mean lethal hit (63 percent reduction in activity). This result is consistent with greater repair of platinum-DNA adducts in the resistant cells.

These results should not be construed to mean that DNA repair is the only mechanism of cisplatin resistance. There is evidence that relative amounts of glutathione are increased in cisplatin-resistant cells.¹⁰⁶ Glutathione presumably uses its thiol moiety to coordinate platinum and diminish the amount that can bind to DNA. Reduced influx or increased efflux of a drug constitutes additional mechanisms by which cells become resistant. Further studies are required to ascertain which of these possibilities is most important for the cisplatin resistance phenomenon.

The discovery that cells can become resistant to cisplatin by repairing DNA lesions suggests a way to explain the selectivity of the drug for certain tumor tissue, and even the selective cytotoxicity of the drug for tumor versus normal cells of the same tissue. Tumor cells that cannot repair platinum-DNA adducts would be most affected by *cis*-DDP. This idea forms one of the central hypotheses about the molecular mechanism of action of *cis*-DDP, details of which can be probed by bioinorganic chemists. Specifically, it is important to inquire what DNA adducts formed by *cis*-DDP are both cytotoxic and repairable, what enzymes are responsible for such repair in mammalian cells, by what mechanisms these enzymes operate, and how this knowledge can be used to design better metal-based antitumor drugs and chemotherapeutic protocols.

(iv). DNA-protein Interactions

Most of the phenomena discussed in this section, inhibition of replication, DNA repair, drug resistance, and mutagenesis, probably involve interaction of a protein or group of proteins with platinated DNA. These interactions are clearly important in determining the biological consequences of DNA templates containing bound platinum. Very recent experiments have uncovered the existence of proteins from a variety of mammalian sources that bind specifically to DNA platinated with *cis*- but not *trans*-DDP.¹⁰⁷ Identifying the nature and function of these factors may provide important clues about the mechanisms of antitumor activity, drug resistance, or repair. Study of protein-DNA-drug interactions is an essential feature of the bioinorganic chemistry of platinum chemotherapeutic agents.

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