9.10: Bioinorganic Chemistry of Platinum Anticancer Drugs; How Might They Work? (Part 2)

6. Effects of DNA Structure on Platinum Binding

a. A-, B-, and Z-DNA[140]

As discussed in more detail in Chapter 8, double-helical DNA can adopt different polymorphic forms depending on the conditions in solution or polycrystalline fiber. Even within a given DNA molecule, there can be sequence-dependent local secondary and tertiary structural differences that constitute important signals for cellular DNA binding and processing molecules. An example already discussed is the recognition of palindromic sequences by type II restriction endonucleases. As shown in Figure 9.24, three such DNA polymorphs are the right-handed A- and B- and the left-handed Z-forms. Most commonly encountered in solution is B-DNA, characterized by well-classified major and minor grooves designated by arrows in Figure 9.24. The targets of platinum binding, guanine N7 atoms, are situated in the major groove.

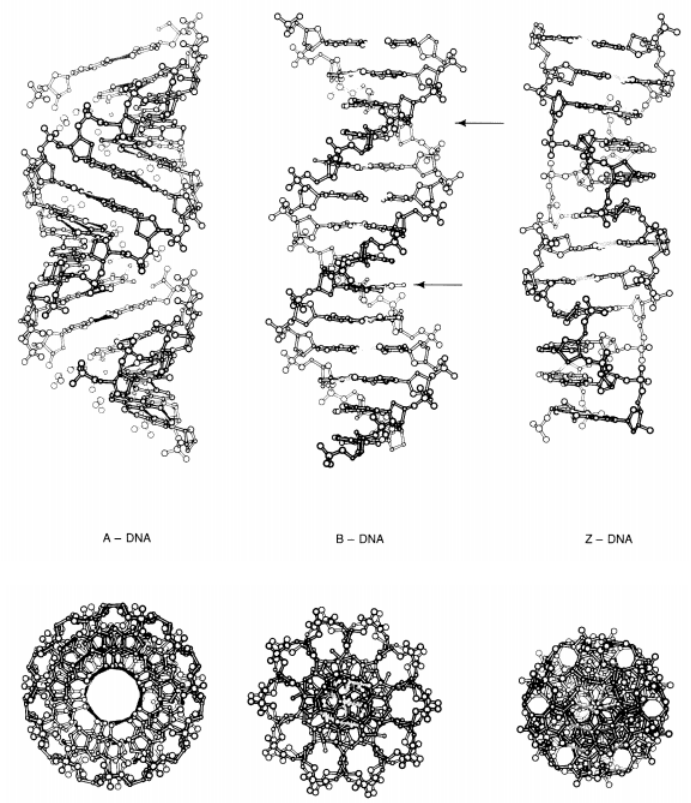

Figure 9.24 - Representations of side (above) and top (below) views of three major classes of double-stranded DNA. For B-DNA, arrows near the top and bottom of the helix designate the minor and major grooves, respectively. Reproduced with permission from Reference 140.

To what extent do sequence-dependent local structural modulations affect platinum binding? Although no general answer to this question can be given, there are several interesting anecdotal pieces of information worth mentioning. Z-DNA, a form favored by alternating purine-pyrimidine sequences such as in poly d(GC), does not constitute a particularly good target for *cis*-DDP binding. For one thing, it lacks the preferred d(GpG) or d(ApG) sequences. The monofunctional $[Pt(dien)Cl]^{+}$ complex, however, facilitates the B-DNA → Z-DNA conformational transition, as demonstrated by circular dichroism and ^{31}P NMR spectroscopic data.[141] In Z-DNA, the guanosine nucleoside adopts the syn conformation (Figure 9.9), which is presumably favored by placing a bulky $\{Pt(dien)\}^{2+}$ moiety on N7. Moreover, the local charge density on DNA is greater in Z- than B-DNA, owing to the closer proximity of the phosphate groups, and the former is presumably stabilized by the +2 charge on the platinum complex.

b. Effects of Local Sequence and of Free and Linked Intercalators on Platinum Binding

Of more interest perhaps to anticancer drug-DNA interactions is the fact that some d(GpG), d(ApG), and even d(GpA) targets for *cis*-DDP binding are very sensitive to the sequences in which they are embedded. This phenomenon was first discovered during

exonuclease III mapping studies of cisplatin binding to a 165-bp restriction fragment from pBR322 DNA.[142] Although *cis*-DDP binding stops the enzyme at G_3, G_5, and GAGGGAG sequences, at a $(D/N)_b$ ratio of 0.05 there is little evidence for coordination to an apparently favored G_6CG_2 sequence (Figure. 9.25, lane 8). When platination was carried out in the presence of the DNA intercalator EtdBr (Figure 9.11), however, the G_6CG_2 sequence became a Pt binding site (Figure 9.25, lanes 9-12). A more extensive exonuclease III mapping study of this phenomenon suggested that d(CGG)-containing sequences in general are less well platinated by *cis*-DDP.[143,144] Moreover, only EtdBr, and not other acridine or phenanthridinium type intercalators, was able to promote an enzyme-detectable *cis*-DDP binding to these sequences. A suggested explanation for these results is that local d(purCGG) sequences might have an A-DNA-type structure (Figure 9.24) in which the major groove is narrow, inhibiting access of platinum to N7 of guanosine nucleosides. In the presence of the intercalator EtdBr, the local DNA structure might be altered in such a manner as to permit binding.[143,144]

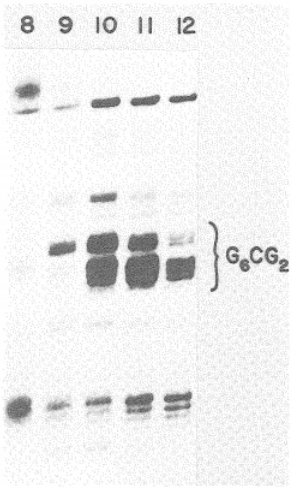

Figure 9.25 - Autoradiograph of Exo III mapping results for cis-DDP binding to a 165-bp DNA restriction fragment. The Pt/nucleotide ratio is 0.05. Lanes 8-12 contain DNA platinated in the presence of 0, 0.012, 0.057, 0.12, and 0.23 Etd/nucleotide, respectively. For more details, see Reference 142.

In accord with this interpretation, and further to delineate a possible reason why acridines and deaminated ethidium cations do not promote cisplatin binding to d(purCGG) sequences, NMR studies were performed that revealed the mean residence time of EtdBr on DNA to be 6 to 21 times longer than that of any of the other intercalators examined.[144] Thus, for these latter intercalators, the local DNA structure presumably can relax back to one unfavored for cisplatin binding before it can diffuse to the site. Moreover, when acridine orange (AO), one of the five intercalators studied that does not promote cisplatin binding to excluded sites, was covalently attached to dichloroethylenediamineplatinum(ll) (Figure 9.26) via a hexamethylene linker chain, the resulting AO-Pt molecule was able to bind to all d(CpGpG) sites, as determined by exonuclease III mapping. In the tethered molecule, the high local concentration near the intercalator binding site facilitates attachment of the $\{Pt(en)\}^{2+}$ moiety to DNA before the acridine orange fragment can diffuse away and the structure can relax to reform the excluded site.

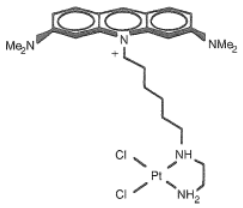

Figure 9.26 - Structure of AO-Pt in which the $\{Pt(en)C_{12}\}$ moiety is linked by a hexamethylene chain to acridine orange.

Subsequently, the excluded site phenomenon was found for *cis*-DDP binding, as assayed by the 3'-5'-exonuclease activity of T4 DNA polymerase.[145] Enzyme stopping sites were observed at all d(GpG) sequences, but only weakly when at a d(GTGGTC) site. Similarly, d(ApG) was not modltled when embedded in pyGAGCpy and pyGAGCA sequences. Although most d(GpA) sequences were not platinated, as detected by T4 mapping, a few were. These results further underscore the importance of local sequence modulation of cisbinding to DNA.

c. DNA-Promoted Reaction Chemistry

In the EtdBr-enhanced binding of *cis*-DDP to DNA, a small fraction (< 5 percent) of the intercalator is strongly bound and can be dialyzed out only very slowly.[145] The detailed structure of this DNA-cisplatin-EtdBr ternary complex has been established, and involves *cis*-$\{Pt(NH_3)_2\}^{2+}$ binding to the exocyclic amino groups of ethidium as well as to donor sites on DNA.[146] This assignment was proved by synthesizing *cis*- $[Pt(NH_3)_2CEtd)CI]^{2+}$ complexes in dimethylformamide solution and then allowing them to react with DNA. The optical spectra of the resulting adducts were identical to that of the ternary complex. The reaction of *cis*-DDP, and DNA to form the ternary complex is promoted by the favorable orientation of the exocyclic amino group of intercalated Etd with respect to the coordination plane of bound to the double helix. The N-8 exocyclic amino group of ethidium, bound intercalatively at a site adjacent to a purine N-7 coordinated *cis*-$\{Pt(NH_3)_2CI\}^{+}$ moiety, is positioned above the platinum atom in a structure resembling the transition state for a square-planar substitution reaction. The structure of this transition state has been modeled in a molecular mechanics calculation (Figure 9.27 See color plate section, page C-16.),[147] and evidence has been obtained that indicates selective binding of platinum to the Etd N-8 amino position.[148]

Since cisplatin is usually administered in combination chemotherapy with other drugs, many of which contain intercalating functionalities, strong covalent, DNA-promoted interactions between drug molecules at a target site must be considered as possibly relevant to the molecular mechanism of action. In such a situation, there must be a strong binding preference for both drug molecules for the same target sequences, since on probability grounds alone it is unlikely that both would migrate to the same site by random diffusion at the low concentrations found *in vivo*.

d. Effects of DNA Function on Platinum Binding

Although there is yet little known about this topic for cisplatin, it is worth pointing out that other DNA-targeted drugs, such as bifunctional alkylating agents, bind preferentially to actively transcribed genes. It is therefore possible that platinum exhibits such preferences, for example, to single-stranded DNA at or beyond the transcription fork, compared to duplex DNA in chromatin structures. Or, perhaps, it too binds selectively to actively transcribed DNA. Investigation of these possibilities seems worthwhile.

7. Speculations About the Molecular Mechanism

a. Is There a Single Mechanism?

Most investigators now agree that DNA is the cytotoxic target of cisplatin. We have seen that the drug inhibits DNA replication by binding to the template and halting the processive action of DNA polymerase. Less well-studied is the inhibition of transcription by platinum-DNA adducts, but recent evidence clearly indicates that they can do so. Studies of the effects of platinum on cells growing in culture reveal that DNA replication and cell growth can continue without cell division in the presence of low levels (1 μg/mL) of *cis*-DDP cells are arrested at the G2 phase, the stage of cell growth just preceding division.[105] G2 arrest was reversible, but at higher cisplatin levels (8 μg/mL), cell death occurred. These observations led to the speculation that, perhaps, post-replication DNA repair can handle the toxicity associated with a platinum-damaged template, at least for DNA synthesis, but that there is no known pathway by which transcription can circumvent Pt-DNA lesions. Possibly, inhibition of transcription is ultimately a more lethal event than inhibition of replication. This idea is inconsistent with the well-established fact that thymidine incorporation into DNA is more affected by low levels of cisplatin than is uridine incorporation into RNA. Might there be more than one biochemical pathway by which cisplatin manifests its anticancer activity? Further work is necessary to address this intriguing question.

b. Is There a "Critical Lesion"?

We now have an excellent understanding of the major DNA adducts made by *cis*-DDP, their structures, and the corresponding DNA distortions. Information about adducts made by the inactive *trans* isomer, though not as complete, is also substantial. During the period when this knowledge was being accumulated, it was of interest to learn whether a "critical lesion," a specific DNA adduct with a unique molecular structure might be responsible for the antitumor activity of the drug. At present, it appears that all bidentate adducts made by *cis*- and *trans*-DDP can inhibit replication, although they may not be equally efficient at doing so.[149] Even monofunctional adducts of the kind formed by *cis*-$[Pt(NH_3)_2(4\text{-Br-py})CI]^{+}$ can block replication.[96] Thus, it be better to think about the concept of "critical lesion" in a functional sense, where the rates of adduct formation, removal, and enzyme inhibition together determine which family of adducts will exhibit antitumor activity and which will not. Here the biochemistry of the host cell will also be an important determinant. Clearly, more studies are required to delineate these possibilities.

c. Replication and Repair in the Tumor Cell[150]

If the anticancer activity of cisplatin arises from damaged DNA templates, then the drug could be selectively toxic to cancer versus normal cells of the same tissue if repair of DNA damage occurred more efficiently in the latter. The best way to study this phenomenon would be to measure the platinum-DNA levels and list the spetrum of adducts formed in tumor versus normal biopsy tissue obtained from patients undergoing cisplatin chemotherapy. As described previously, methodologies are now reaching the point where such experiments can be carried out in order to test the key hypotheses about the mechanism of action of cisplatin. In addition, powerful new methods have recently been developed to screen for DNA bmdlnlg proteins. If one could identify proteins that bind selectively to *cis*-DDP-platinated DNA and determine function, further insights into cellular replication and repair phenomena would be forthcoming. Such cellular factors that bind selectively to DNA containing cisplatin adducts have, in fact, recently been discovered.[107] The experiments that led to this finding and their possible implications for the molecular mechanism of cisplatin are described in the next section.

d. Structure-Specific (or Damage) Recognition Proteins[150]

If selective repair of platinum-DNA adducts in cells of different origin is an integral part of the anticancer mechanism of *cis*-DDP, then it is important to identify the cullular factors associated this phenomenon. In bacteria, *cis*-DDP adducts on DNA are removed by excision repair, a process in which the lesion is first identified and then excised by the uvrABC excinuclease system.[151] In this process, the uvrA protein first binds to the adducted DNA. Subsequently, the uvrB and C proteins excise the damaged strand, which additional cellular proteins rebuild by copying the genetic information from the remaining strand.

The repair of *cis*-DDP intrastrand crosslinks in mammalian cells is much less well understood. Under the assumption that an analogue of the uvrA protein might exist in such cells, experiments were carried out to try to isolate and clone the gene for such a protein. In particular, the mobility of platinated DNA restriction fragments of defined length was found to be substantially retarded in electrophoresis gels following incubation extracts from human HeLa cells.[107] This gel-mobility shift was attributed to the binding of factors termed "damage recognition proteins" (or DRPs). Subsequent studies with site-specifically platinated oligonucleotides (see V.D.8) revealed that the cisplatin DRP binds specifically to DNA containing the intrastrand *cis*-[$Pt(NH_3)_2${d(pGpG)}] or *cis*-[$Pt(NH_3)_2${d(pApG)}] crosslink. In parallel work, the gene encoding for a DRP was cloned[152] and used to demonstrate the occurrence of such a in nearly all eukaryotic cells. Since binding of the DRP to platinated DNA is not specific for the ammine ligands opposite the crosslinked nucleobases, the interaction is thougllt to involve recognition of local changes in the twist and bending of the double helix. Figure 9.28 depicts one possible structure for the complex formed between *cis*-DDP platinated DNA and a DRP. More recently, the cloned proteins were found to contain a high mobility group (HMG) protein box, and even HMG 1 itself binds to cisplatin-modified DNA.[152] The class of proteins was renamed "structure-specific recognition proteins" (SSRPs).

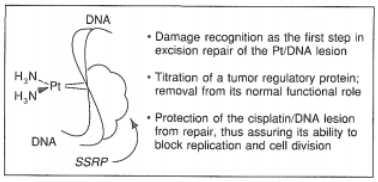

Figure 9.28 - Model depicting the binding of an SSRP to cisplatin-damaged DNA and several hypotheses for its role in the molecular mechanism.

The discovery of SSRPs that bind specifically to cisplatin-modified DNA raises several questions that are the subjects of current study. The first is to determine whether the proteins are an integral component in the mechanism of action of the drug. Although it has not yet been possible to induce the proteins by treating cells with cisplatin, nor have elevated or suppressed levels been found in platinum-resistant cells, deletion of an SSRP gene in yeast has re(~enltlv afforded a mutant strain less sensitive to cisplatin than wild-type cells.[153] This result links a yeast SSRP with cellular sensitization to the drug. Such a protein could contribute to the molecular mechanism in one of several ways (Figure 9.28). It might be the analogue of uvrA, which, as mentioned above, recognizes damage and signals the cell to perform excision repair. If so, then one would like to depress the levels of the protein in cancer cells to make them more sensitive to the drug. A second possibility is that the true role of the SSRP is to serve as a tumor-cell activator, and that cisplatin lesions titrate it away from its functionally active sites on the DNA. Alternatively, binding of the protein could protect cisplatin adducts from repair, preserving their lethality at the time of cell division and leading to the arrest of tumor growth. This last hypothesis would require more of the SSRP in cells sensitive to the drug. Studies are currently in progress to delineate these three and other hypotheses, and to learn whether the discovery of the SSRPs has heralded the final chapter in the quest for the molecular mechanism of cisplatin or merely been an entertaining sidelight.

e. Drug Resistance: What Do We Know?[150]

Perhaps the most serious problem for successful chemotherapy of tumors is drug resistance.[102] In most tumors there exists a subpopulation of cells that are naturally resistant to a given drug; as the sensitive cells are killed, these refractory clones take over. In addition, resistance can be acquired by tumor cells following repeated application of the drug. Attempts to identify mechanisms responsible for cisplatin resistance have therefore been the subjects of considerable research activity. Other DNA-damaging agents sometimes amplify genes as a mechanism of drug resistance. An example is the multidrug resistance phenomenon, in which a gene encoded for a P-glycoprotein is amplified in cells resistant to agents such as daunomycin. This protein is believed to increase efflux of the drug through the cell membrane by an ATP-dependent, energy-driven pump. There is currently an intensive search underway to see whether the cisplatin resistance phenomenon has a genetic origin. If a cisplatin resistance gene could be cloned and its phenotype identified, a powerful new avenue would be opened to overcome drug resistance.
