9.9: Bioinorganic Chemistry of Platinum Anticancer Drugs- How Might They Work?

The material in this section constitutes the major portion of this chapter. One important goal of the discussion is to illustrate, by means of an in-depth analysis of a single case history, the questions that must be addressed to elucidate the molecular mechanism of an inorganic pharmaceutical. Another is to introduce the techniques that are required to answer these questions, at least for the chosen case. The inorganic chemist reading this material with little or no biological background may find the experience challenging, although an attempt has been made to explain unfamiliar terms as much as possible. It is strongly advised that material in Chapter 8 be read before this section. Toward the end of this section, the results obtained are used to speculate about a molecular mechanism to account for the biological activity of the drug. Experiments directed toward evaluating the various hypotheses are delineated. Once the mechanism or mechanisms are known, it should be possible to design new and better antitumor drugs which, if successful, would be the ultimate proof of the validity of the hypotheses. This topic is discussed in the next and final section of the chapter. Such an analysis could, in principle, be applied to probe the molecular mechanisms of the other metals used in medicine described previously. In fact, it is hoped that the approach will prove valuable to students and researchers in these other areas, where much less information is currently available at the molecular level.

The material in this section has been organized in the following manner. First we discuss the relevant inorganic chemistry of platinum complexes in biological media. Next we summarize the evidence that DNA is a major target of cisplatin in the cancer cell, responsible for its antitumor activity. The chemical, physical, and biological consequences of damaging DNA by the drug are then described, followed by a presentation of the methodologies used to map its binding sites on DNA. The detailed structures of the DNA adducts of both active and inactive platinum complexes are then discussed, together with the way in which the tertiary structure of the double helix can modulate these structures. Finally, the response of cellular proteins to cisplatin-damaged DNA is presented, leading eventually to hypotheses about how tumor cells are selectively destroyed by the drug. Together these events constitute our knowledge of the' 'molecular mechanism," at least as it is currently understood.

1. Reactions of *cis*-DDP and Related Compounds in Aqueous, Biological, and Other Media

cis-Diamminedichloroplatinum(II) is a square-planar d^8 complex. As such, it belongs to a class of compounds extensively investigated by coordination chemists.[55] Typically, such compounds are relatively inert kinetically, do not usually expand their coordination numbers, and undergo ligand substitution reactions by two independent pathways with the rate law as given by Equation (9.4). The rate constants k_1 and k_2 correspond to first-order (solvent-assisted) and second-order (bimolecular) pathways;

$$Rate = (k_1 + k_2[Y])[complex] \tag{9.4}$$

[Y] is the concentration of the incoming ligand. Usually, $k_1 << k_2$ by several orders of magnitude. In biological fluids, however, the concentration of a potential target molecule could be ~10^{-6} M, in which case $k_1 \simeq k_2$[Y]. Substitution of ligands in *cis*-DDP, required for binding to a cellular target molecule, is therefore likely to proceed by the solvent-assisted pathway. Such a pathway is assumed in the ensuing discussion.

For the hydrolysis of the first chloride ion from *cis*- or *trans*-DDP,

$$[Pt(NH_3)_2Cl_2] + H_2O \rightleftharpoons [Pt(NH_3)_2Cl(OH_2)]^+ + Cl^- \tag{9.5}$$

the k_1 values at 25 °C are 2.5 x 10^{-5} and 9.8 x 10^{-5} s^{-1}, respectively.[55] These hydrolyzed complexes can undergo further equilibrium reactions, summarized by Equations (9.6) to (9.9).

$$[Pt(NH_3)_2Cl(OH_2)]^+ \rightleftharpoons [Pt(NH_3)_2Cl(OH)] + H^+ \tag{9.6}$$

$$[Pt(NH_3)_2Cl(OH_2)]^+ + H_2O \rightleftharpoons [Pt(NH_3)_2(OH_2)_2]^{2+} + Cl^- \tag{9.7}$$

$$[Pt(NH_3)_2(OH_2)_2]^{2+} \rightleftharpoons [Pt(NH_3)_2(OH_2)(OH)]^+ + H^+ \tag{9.8}$$

$$[Pt(NH_3)_2(OH_2)(OH)]^+ \rightleftharpoons [Pt(NH_3)_2(OH)_2] + H^+ \tag{9.9}$$

The formation of dimers such as $[Pt(NH_3)_2(OH)]_2^{2+}$ and higher oligomers can also occur,[56,57] but such reactions are unlikely to be important at the low platinum concentrations encountered in biological media, Reactions (9.5) to (9.9), which depend on pH and the chloride-ion concentrations, have been followed by ^{195}Pt (I = $\frac{1}{2}$, 34.4 percent abundance) and ^{15}N (using enriched compounds) NMR spectroscopy. The latter method has revealed for the *cis*-diammine complexes pK_a values of 6.70 ± 0.10 at 25 °C for Reaction (9.6) and of 5.95 ± 0.1 and 7.85 ± 0.1 at 5 °C for Reactions (9.8) and (9.9), respectively.[58]

The effects of pH and Cl^- ion concentration on the species distribution of platinum compounds have been used to fashion the following plausible argument for the chemistry of *cis*-DDP *in vivo*.[59] With the use of thermodynamic data for the ethylenediamine (en) analogue $[Pt(en)Cl_2]$ the relative concentrations of hydrolyzed species at pH 7.4 were estimated (see Table 9.3) for blood plasma and cytoplasm (Figure 9.7). The higher chloride ion concentration in plasma preserves the complex as the neutral molecule cis-DDP, which passively diffuses across cell membranes. The lower intracellular chloride ion concentration facilitates hydrolysis reactions such as Equations (9.5) to (9.9), thereby activating the drug for binding to its biological target molecules. There is, of course, a reasonable probability that *cis*-DDP and species derived from it will encounter small molecules and macromolecules *in vivo* that divert it from this route to the target. We have already seen such cases; cisplatin binds to serum proteins, and there is good evidence that intracellular thiols react with the drug.[60] Glutathione, for example, is present in millimolar concentrations in cells. How, one might ask, does cisplatin swim through such a sea of sulfur donors to find its target in the tumor cell? Is it possible that a modified form of the drug, in which a Pt-CI bond has been displaced by thiolate to fonn a Pt-S bond, is the actual species responsible for its activity? Although these questions have not yet been satisfactorily answered, there is reason to believe that such reactions are not directly involved in the molecular mechanism of action. As evident from structure-activity relationship studies, the most active compounds have two labile ligands in *cis* positions. If Pt-S bonds were required, then compounds already having such linkages would be expected to exhibit activity and they do not. Rather, it seems most likely that the antitumor activity of cisplatin results from surviving species of the kind written in Equations (9.5) to (9.9) that find their way to the target molecule, and that the induced toxicity must arise from a significantly disruptive structural consequence of drug binding. Since only *cis* complexes are active, it is reasonable for the coordination chemist to infer that the stereochemistry of this interaction is of fundamental importance.

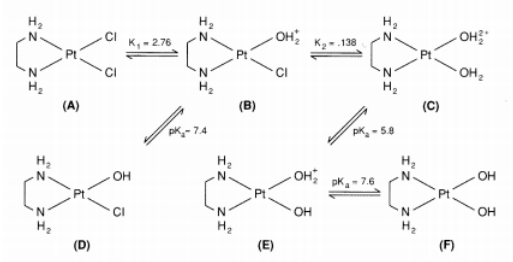

Figure 9.7 - Hydrolysis reactions of antitumor platinum complexes and an estimate of the species present in plasma and cytoplasm (reproduced by permission from Reference 51).

Table 9.3 - Distribution of various adducts formed between *cis*-DDP or $[^3H][Pt(en)Cl_2]$[a] and DNA *in vitro* and *in vivo*.[118-122]

a) A radiolabeled analogue of *cis*-DDP, [^{3}H]dichloroethylenediamineplatinum(II).

b) A_2 represents either $(NH_3)_2$ or ethylenediamine.

c) By difference.

d) Percentage of adducts based on total amount of platinum eluted from the separation column.

e) Percentage of adducts based on total amount of radioactivity eluted from the separation column.

f) Not given.

g) Chinese hamster ovary cells treated with 83 μM *cis*-DDP.

h) Results from ELISA.

i) Results from AAS. Where the signal was too weak for reliable quantitation, the maximal amount possible is given. Adapted from Table 1 in Reference 81.

D/N Ratio	Total Incubation Time	Adducts		Formed		
		cis-$[PtA_2\{d(pGpGl\}]$[b]	*cis*-$[PtA_2\{d(pApG\}]$[b]	*cis*-$[PtA_2\{d(GMP)\}_2]$[b]	Mono-functional Adducts	Remaining Platinum[c]
In vitro						
0.055[c]	5 h (50 °C)	47-50%	23-28%	8-10%	2-3%	10%
0.022[d]	5 h (50 °C)	60-65%	20%	~4%	~2%	9-14%
0.01[e]	16 h (37 °C)	62%	21%	7%	—	10%

D/N Ratio	Total Incubation Time	Adducts Formed				
		cis-[PtA_2{d(pGpGl}][b]	*cis*-[PtA_2{d(pApG}][b]	*cis*-[PtA_2{d(GMP)}$_2$][b]	Mono-functional Adducts	Remaining Platinum[c]
[ef]	30 m (37 °C)	36%	3%	8%	40%	13%
[ef]	2 h (37 °C)	54%	9%	9%	14%	14%
[ef]	3 h (37 °C)	57%	15%	9%	4%	15%
In vivo						
[dg]	1 h (37 °C)	35.9 ± 4.7%[h]	<34%[i]	3.1 ± 1.6%[h]	38.5%[i]	~22%
[dg]	25 h (37 °C)	46.6 ± 6.8%[h]	<48%[i]	3.0 ± 0.9%[h]	<14.5%[i]	~50%

Reactions of platinum compounds with components in media used to dissolve them can give and undoubtedly have given rise to misleading results, both in fundamental mechanistic work and in screening studies. A particularly noteworthy example is dimethylsulfoxide (DMSO), which even recently has been used to dissolve platinum compounds, presumably owing to their greater solubility in DMSO compared to water. As demonstrated by ^{195}Pt NMR spectroscopy, both *cis*- and *trans*-DDP react rapidly ($t_{1/2}$ = 60 and 8 min at 37 °C, respectively) to form $[Pt(NH_3)_2CI(DMSO)]^+$ complexes with chemical and biological reactivity different from those of the parent ammine halides.[61]

2. Evidence that DNA is the Target

Two early sets of experiments pointed to interactions of cisplatin with DNA, rather than the many other possible cellular receptors, as an essential target responsible for cytotoxicity and antitumor properties.[62,63] Monitoring the uptake of radiolabeled precursors for synthesizing DNA, RNA, and proteins, showed that [^{3}H]thymidine incorporation was most affected by therapeutic levels of cisplatin for both cells in culture and Ehrlich ascites cells in mice. Since independent studies showed that *cis*-DDP binding to DNA polymerase does not alter its ability to synthesize DNA, it was concluded that platination of the template and not the enzyme was responsible for the inhibition of replication.

In a second kind of experiment demonstrating that DNA is a target of cisplatin, hydrolyzed forms of the drug in low concentrations were added to a strain of *E. coli* K12 cells containing a sex-specific factor F.[64,65] After free platinum was removed, these F^+ cells were conjugated with a strain of *E. coli* K12 cells lacking this factor that had previously been infected with lambda bacteriophage. Addition of *cis*-DDP, but not *trans*-DDP, directly to the latter infected F^- cells had been shown in a separate study to accelerate cell lysis. Conjugation with the platinum-treated F^+ cells produced the same effect, strongly suggesting that Pt had been transferred from the F^+ to the F^- cells. Since only DNA is passed between the F^+ and F^- strains, it was concluded that Pt was attached to the DNA and that this modification was essential for the observed lysis of the cell. Further studies showed a good correlation between cell lysis by platinum compounds and their antitumor properties.

Various other observations are consistent with the notion that platinum binding to DNA in the cell is an event of biological consequence.[66] The filamentous bacterial growth observed in the original Rosenberg experiment is one such piece of evidence, since other known DNA-damaging agents, for example, alkylating drugs and x-irradiation, also elicit this response. Another is the greater sensitivity toward *cis*-DDP of cells deficient in their ability to repair DNA. Finally, quantitation of the amount of platinum bound to DNA, RNA, and proteins revealed that, although more Pt was bound to RNA per gram biomolecule, much more Pt was on the DNA when expressed as a per-molecule basis. In the absence of any selective interaction of Pt with a specific molecule, only one out of every 1,500 protein molecules (average M.W. ~60 kDa) in a cell will contain a single bound platinum atom, whereas hundreds or thousands of Pt atoms are coordinated to DNA (M.W. ~10^{11}). If the replication apparatus cannot bypass these lesions, then cell division will not occur, and tumor growth is inhibited.

Although these and other results all point to DNA as an important cellular target of cisplatin, most likely responsible for its anticancer activity, this information does not explain why tumor cells are more affected by *cis*-DDP than non-tumor cells of the same tissue. Moreover, why is *trans*-DDP, which also enters cells, binds DNA, and inhibits replication, albeit at much higher doses (see discussion below), not an active anticancer drug? What causes cisplatin to kill cells and not merely to arrest tumor growth? The latter can be explained by DNA synthesis inhibition, but not necessarily the former. Very recent studies have begun to address

these questions using powerful new methodologies of molecular and cell biology, as described in subsequent sections of this chapter. The results, although preliminary, continue to point to DNA as the most important cellular target of cisplatin.