Coordination Chemistry Reviews, 100 (1990) 293–322
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

THE COORDINATION CHEMISTRY OF PLATINUM ANTICANCER DRUGS AND RELATED COMPOUNDS WITH DNA

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(Received 11 July 1989)

CONTENTS

A. INTRODUCTION

The use of coordination compounds as biological probes represents one of the most successful applications of bioinorganic chemistry. Such probes utilize the unique physical properties of metal complexes to serve as "reporters", especially when embedded in the largely organic matrix of most biological systems. Important examples of inorganic probes include paramagnetic shift reagents employed in (NMR) imaging [1], heavy metal complexes used in macromolecular X-ray crystallography [2,3] redox-active compounds used to study biological electron transfer [4–6], radioactive imaging agents [7–10], DNA footprinting reagents [11–15] and numerous examples of the introduction of spectroscopically active metals into proteins

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and metalloenzymes [16–19]. Successful application in each of these cases requires selective localization, a limitation that emphasizes the need for greater understanding of molecular recognition between inorganic complexes and biological macromolecules. Nevertheless, inorganic probes now make at least as great a contribution in revealing structure and function in biological systems as their organic counterparts.

In contrast, relatively few coordination compounds have been developed as pharmaceuticals, owing largely to the biomimetic and serendipitous nature of drug development. While many organic pharmaceuticals have evolved from natural products, nature has provided relatively few inorganic analogs. Considerable research has focused on such examples that exist, however, as in the study of the iron-based antitumor agent bleomycin and its derivatives [20,21]. General unfamiliarity with inorganic chemistry within the pharmaceutical industry has also favored testing and development of organic compounds as drugs. Once again, however, there is substantial interest in those cases where the therapeutic activities of coordination compounds have been discovered, often by accident, as in the case of the leading antitumor drug *cis*-diamminedichloroplatinum(II) (*cis*-DDP) [22–28].

Other important examples of inorganic-based pharmaceuticals include metallocene antitumor complexes [29,30], gold antiarthritic compounds [31–33] and lithium antidepressants [34–36]. In all these cases, research is largely focused on elucidating the currently unknown mechanisms of action of these complexes. Attempts to generate new and better inorganic drugs have usually involved synthesis of derivatives of compounds known to be active, the most extensive examples being the numerous platinum and other late transition metal analogs of *cis*-DDP [37–39]. Some attempts have also been made to use nuclear properties of inorganic compounds for therapy, for example in the tumor-specific generation of radiation by Mössbauer excitation of ^{57}Fe-bleomycin [40] or by neutron activation of ^{10}B cluster compounds [41]. In addition, several coordination compounds are being prepared and evaluated as radiosensitizers in the treatment of cancer [42,43].

In principle, coordination compounds offer a great variety of shapes and reactivities for use in drug design. Exploiting this potential, however, requires an understanding, at the molecular level, of how inorganic complexes can recognize, bind and perturb biological macromolecules. This article reviews the considerable progress being made in understanding the interactions of one class of coordination compounds, those of platinum(II), with a specific macromolecular receptor, DNA. Interest in such Pt–DNA interactions has been stimulated by the successful use of platinum complexes as probes of DNA structure and by the remarkable antitumor activity of certain platinum compounds that act at the DNA level. These studies

exemplify the multidisciplinary aspects of this kind of bioinorganic research, and illustrate many of the principles involved in studying interactions between coordination compounds and biological macromolecules.

B. HISTORICAL INTEREST IN Pt–DNA INTERACTIONS

Platinum(II) coordination compounds were early and important examples of transition metal complexes. Their square-planar geometry was first determined in the classic isomer-counting experiments of Werner [44]. Platinum(II) complexes were subsequently used extensively in studies to determine the mechanisms of square-planar substitution reactions, since they react at convenient rates in aqueous solution and are relatively stable in the +2 oxidation state [45]. Platinum binding to nucleic acids was first investigated in part with the aim of developing heavy metal stains for electron microscopic (EM) studies of DNA. The high electron density of platinum complexes has also led to their use as isomorphous replacement agents for solving macromolecular crystal structures. Examples of these applications include the still common visualization of DNA by platinum shadowing in EM studies [46] and the use of platinum(II) complexes in determining some of the most important nucleic acid crystal structures solved to date, namely $tRNA^{Phe}$ [47], the nucleosome core particle [48] and the first complex of a restriction enzyme with a DNA substrate [49].

Discovery of the antitumor activity of *cis*-DDP further fueled an interest in the aqueous coordination chemistry of platinum and in the study of Pt–DNA interactions. Subsequent investigations have led to a number of advances in basic platinum chemistry including the characterization of previously unknown Pt–aqua and Pt–hydroxo complexes [50–52], platinum blues and other polymeric mixed-valence platinum complexes [53–59], Pt–ascorbate complexes [60,61] and many novel Pt–nucleobase complexes [62]. Increased activity in the field has also led to a greater understanding of the chemistry of platinum in the +3 oxidation state [63] and the chemistry of platinum with biologically relevant sulfur ligands [64–67]. Although these topics are beyond the scope of this review, they serve to illustrate how much fundamental inorganic coordination chemistry may still be discovered when a biological application sparks interest in the bioinorganic chemistry of a transition element.

C. PLATINUM ANTITUMOR DRUGS

(i) DNA as the target

Coordination chemists have long been fascinated with the fact that *cis*-DDP (or cisplatin) is active as an anticancer drug while the trans isomer

is not. Such a difference implies a stereochemical preference for interaction of the cis isomer to biological targets that is not shared by *trans*-DDP. The biomolecule widely accepted as the target of cisplatin is DNA, which reduces the problem to understanding differences in the stereochemistry of *cis*- and *trans*-DDP adducts with DNA.

Evidence that *cis*-DDP exerts biological activity at the DNA level came first from studies demonstrating that *cis*-$[Pt(NH_3)_2Cl_2]$ persistently inhibits DNA synthesis to a greater extent than it affects RNA or protein synthesis [68]. Further support for the hypothesis that cisplatin acts at the DNA level was derived from observations that prokaryotic and eukaryotic cells deficient in DNA repair are often far more sensitive to *cis*-DDP than their parental lines [69–73]. These experiments suggested that the cytotoxicity of the drug is reduced by the removal of *cis*-DDP–DNA adducts. This conclusion was further supported by observations that *cis*-DDP-resistant tumor cells showed an increased rate of Pt–DNA adduct loss compared with non-resistant lines [74–76].

DNA offers a wide variety of potential metal binding sites for platinum owing to its size and chemical complexity [77]. Moreover, although the kinetics and mechanisms of nucleophilic substitution reactions of platinum(II) ammine halides are well understood, there was no a priori reason to suppose that they would apply to the binding of *cis*- and *trans*-DDP to DNA.

(ii) Kinetics of binding to DNA

As shown in Fig. 1, ^{195}Pt NMR spectroscopy has been used to study the binding of *cis*- and *trans*-DDP to short duplex DNA fragments (30–50 base pairs, average molecular weight (MW) $\approx$ 25 000) [78]. The reaction first involves replacement of chloride ion by water in a rate-determining solvolysis step. Binding to DNA to form monofunctional adducts then rapidly ensues. Closure to form bifunctional adducts is kinetically controlled by loss of the second chloride ion, for which $t_{1/2}$ values of 2.1 and 3.1 h have been measured for *cis*- and *trans*-DDP respectively, under the same conditions. Owing to the large chemical shift range for ^{195}Pt, it is possible to follow these individual steps.

(iii) Regioselectivity and structures of DNA adducts

Although the rate and mechanism of formation of DNA adducts of *cis*- and *trans*-DDP do not differ appreciably, the regiospecificity and stereospecificity for the two complexes are dramatically different. In particular, *cis*-DDP prefers to form 1,2-intrastrand crosslinks between adjacent nucleo-

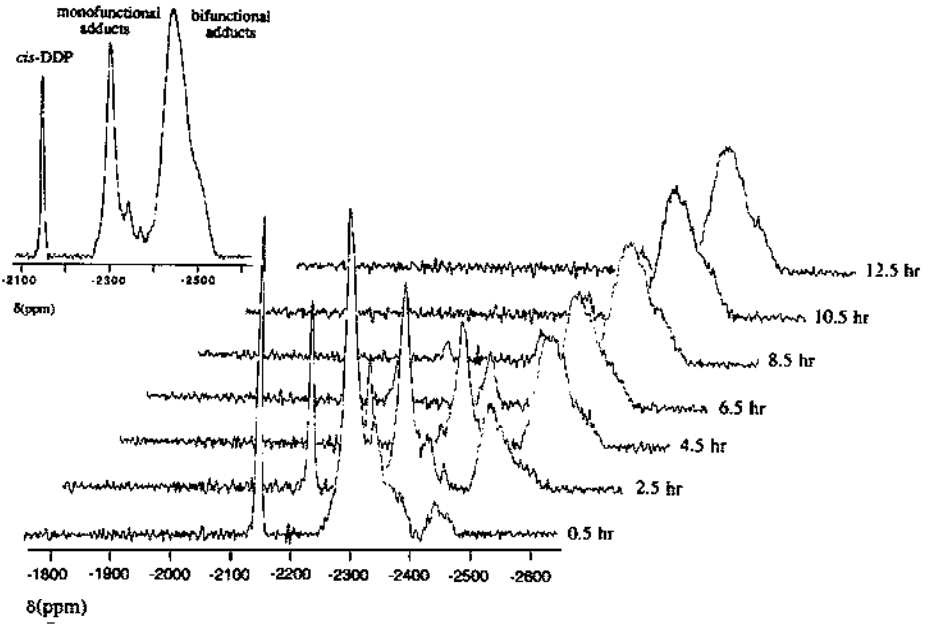


Fig. 1. ^{195}Pt NMR spectra taken during the reaction of *cis*-DDP with double-stranded DNA from chicken erythrocytes. The inset shows the sum of individual spectra (reproduced from ref. 78).

tide bases, with 90% of the adducts involving platinum coordinating to the N7 positions of two guanine bases or an adenine and a guanine base [24]. The structure of the most prevalent adduct, as determined by X-ray crystallography, is depicted in Fig. 2 [79]. Both molecular mechanics model building [80] and NMR [80–82] studies of short oligonucleotide fragments, however, reveal that the trans isomer cannot form an analogous 1,2-intrastrand cross-link. This result is interesting from the viewpoint of a coordination chemist since such an adduct involves a 17-membered chelate ring (Fig. 2) which, because of the stereochemical requirements of the DNA chain, is not large enough to span the trans positions of the *trans*-$\{Pt(NH_3)_2\}^{2+}$ moiety. Instead, the trans isomer forms a variety of DNA adducts [83], including 1,3-intrastrand crosslinks, several of which have now been synthesized and characterized by NMR spectroscopy [81,82,84]. A schematic view of one such structure is given in Fig. 3. As can be seen from the figure, two purine N7 atoms are linked by platinum to close a 23-membered ring. In one instance, involving the dodecanucleotide sequence d(TCTACGCGTTCT), the 1,3-intrastrand *trans*-$\{Pt(NH_3)_2\}^{2+}$ (N7-G(6), N7-G(8)) adduct was found to be metastable, rearranging with a half-life of 47 h at 37°C to afford an equilibrium mixture containing the more stable 1,4-intrastrand N3-C(5), N7-G(8) crosslink [85].

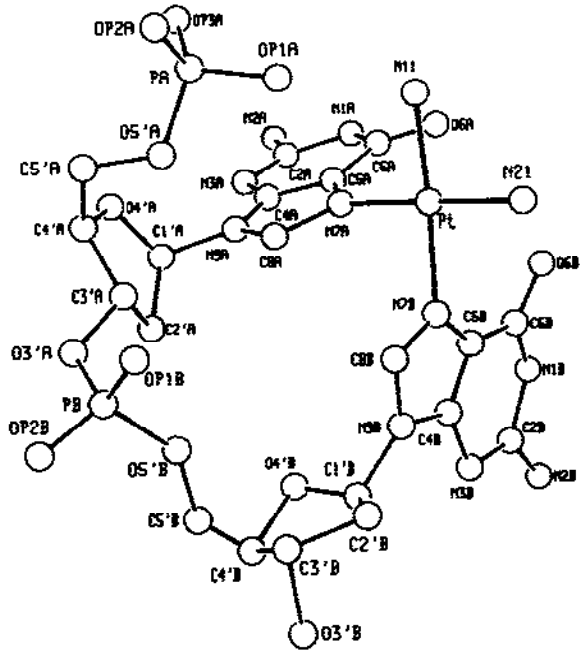


Fig. 2. Structure of the major adduct of *cis*-DDP with DNA, *cis*-[$Pt(NH_3)_2$(d(pGpG)}] (reproduced with permission from S.E. Sherman, D. Gibson, A.H.-J. Wang and S.J. Lippard, Science, 230 (1985) 412).

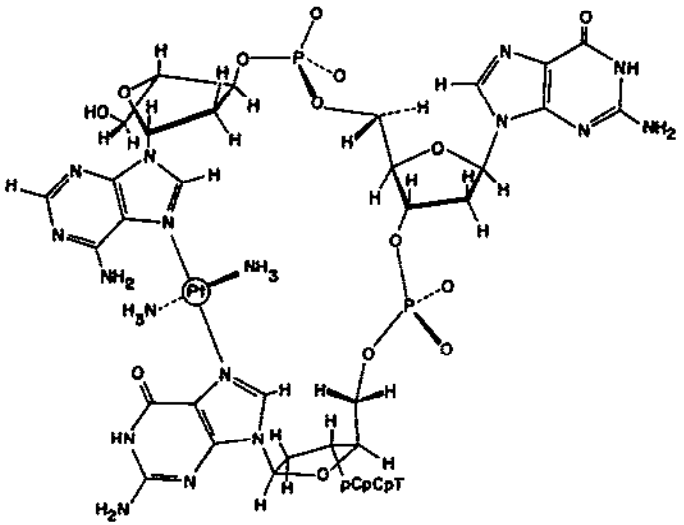


Fig. 3. Structure of the major adduct formed by *trans*-DDP with the oligonucleotide d(ApGpGpCpCpT) (reproduced with permission from ref. 84).

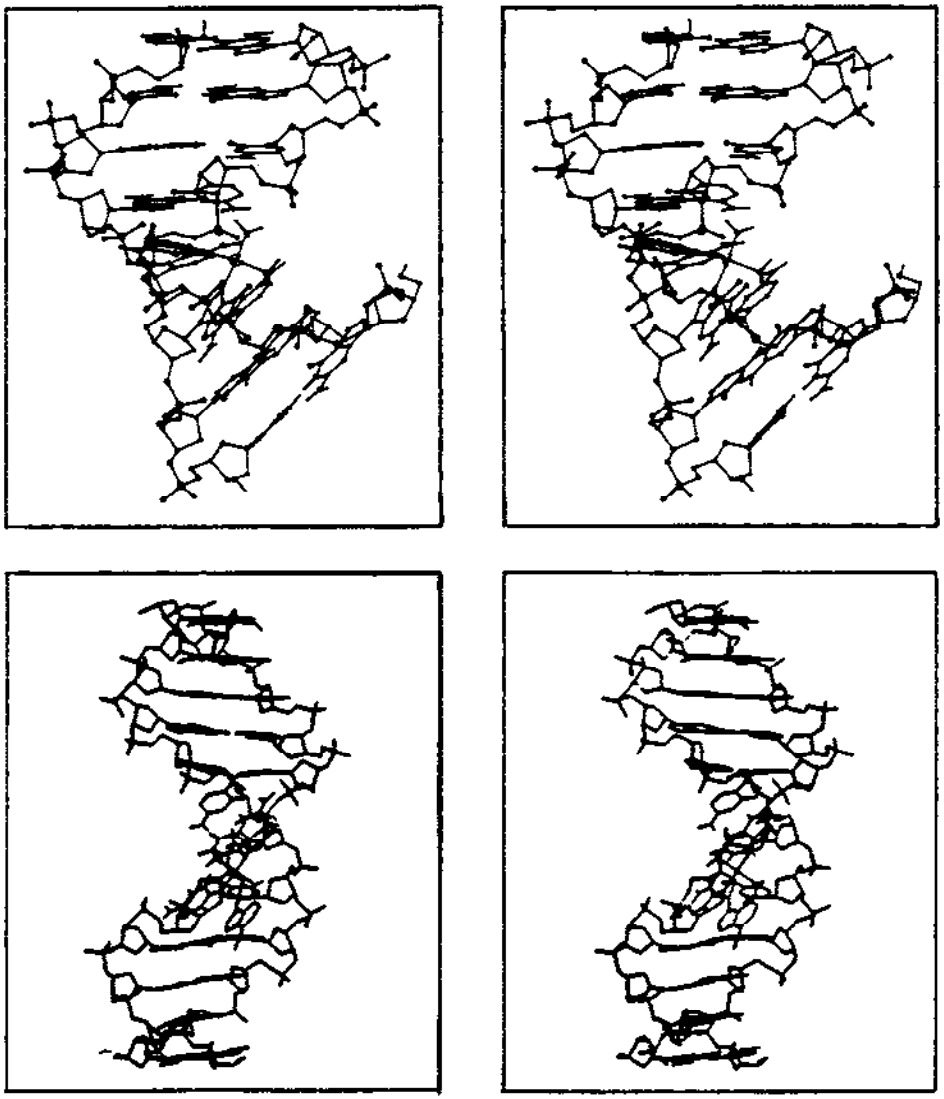


Fig. 4. Molecular mechanics models of (top) *cis*- and (bottom) *trans*-DDP adducts with duplex oligonucleotides (for details, see ref. 80).

Although detailed structural information about *cis*- and *trans*-DDP adducts in double-stranded DNA is not yet available, molecular mechanics and dynamics models have provided insight into the various possibilities. The structures of two principal adducts of *cis*- and *trans*-DDP with DNA, as predicted by molecular mechanics modeling, are depicted in Fig. 4. For *cis*-DDP, both linear and bent DNA molecules containing the *cis*-

$\{Pt(NH_3)_2\}^{2+}$ d(GpG) adduct can be built [86], but recent studies of duplex oligonucleotides containing this and the related *cis*-$\{Pt(NH_3)_2\}^{2+}$ d(ApG) adduct reveal the bent structure to be correct [87,88]. Specifically, platinum binding in this manner kinks the duplex by 32–34°. In the case of *trans*-DDP, a very stable duplex structure has been constructed and refined by molecular dynamics calculations [80]. The two ammine ligands form a hydrogen bonding bridge between the two strands of the DNA, and the intervening nucleotide stacks into the minor groove. It has not been possible to estimate a bending angle for the *trans*-DDP adduct, however, since the platinum appears to be the focus for a hinge joint rather than the directed bend induced by cisplatin [88]. An important objective for the near future is to obtain experimental evidence for the actual structures of adducts formed by the two isomers with DNA, preferably by X-ray diffraction.

(iv) Mechanism of action studies

Of major importance in elucidating the molecular mechanism of cisplatin is the evaluation of the processing of specific adducts with DNA in vivo and the comparison of the information so obtained with their anticancer effects. The relative biological activities of different *cis*-DDP–DNA adducts have thus far been difficult to determine since most studies have been performed on DNA containing all possible *cis*-DDP adducts. It seems likely, however, that only the two major adducts formed by *cis*-DDP will be important in inhibiting DNA replication. The low level of bound drug required to inhibit replication of, for example, a platinated SV40 genome (50% inhibition at about 4 Pt per genome) implies that low frequency adducts, such as the interstrand and intrastrand *cis*-[$Pt(NH_3)_2${d(GpNpG)}] crosslinks, should not even be present on most of the SV40 molecules when replication is inhibited [89,90]. Similarly low levels of bound *cis*-DDP also inhibit the replication of plasmid-sized DNA molecules in other systems [91], again implying that the two major *cis*-DDP adducts, *cis*-[$Pt(NH_3)_2${d(GpG)}] and *cis*-[$Pt(NH_3)_2${d(ApG)}], are largely responsible for inhibition of DNA replication in these systems.

There is no reason a priori to expect different *cis*-DDP–DNA adducts to have identical biological activities. For example, different adducts are not equally mutagenic. *cis*-DDP causes predominantly single base substitution mutations in *E. coli*, although frameshift mutations are also observed [92–95]. Several studies have demonstrated the variable activity of different adducts, implicating either *cis*-[$Pt(NH_3)_2${d(GpNpG)}] [94] or *cis*-[$Pt(NH_3)_2${d(ApG)}] [95] as the most mutagenic species.

Recently, a combination of coordination chemistry and molecular biology has been used to construct DNA molecules containing specific *cis*- and

trans-DDP adducts [96–98] that can subsequently be evaluated for their ability to be replicated or repaired in vitro or in vivo. Engineering of specific *cis*-DDP adducts into DNA in vitro should facilitate more rigorous comparison of different biological activities of the various *cis*-DDP–DNA adducts. Moreover, such systems allow assessment of the effects of platinum binding to DNA without complications arising from platinum modification of other biomolecules [96–102]. In one experiment, a *cis*-[$Pt(NH_3)_2${d(GpG)}] adduct was introduced into a specific site in the plus strand of the *E. coli* bacteriophage M13 [96]. The single platinum adduct reduced the transformation efficiency of the single-stranded phage DNA by 90% compared with an unmodified M13 control, presumably by inhibiting the first round of DNA replication. Similarly, *cis*-DDP modification of purified SV40 DNA inhibits subsequent DNA replication in an in vitro system at biologically relevant drug-to-nucleotide levels [103].

Although the intrastrand crosslinks formed by *cis*- and *trans*-DDP differ substantially, both inhibit DNA replication in vitro and in vivo [25,103]. The cis isomer, however, is far more toxic and mutagenic to cells in culture than *trans*-DDP. In order to obtain equitoxic amounts of the trans isomer bound to DNA, far greater quantities must be added to the culture media. Two possible explanations have been suggested to account for these results. One is that adducts formed in vivo by *trans*-DDP inhibit DNA replication less efficiently than those of *cis*-DDP. Perhaps these adducts are monofunctional, since bifunctional adducts of the two isomers are equally effective at inhibiting replication [25,103]. Monofunctional adducts of *trans*-DDP with DNA may react faster with cellular components, especially those containing sulfhydryl groups such as glutathione. From ^{195}Pt NMR experiments it has been established that glutathione reacts much more rapidly with *trans*-DDP monofunctional adducts than with those formed by cisplatin [78]. Such reactions might deactivate *trans*-DDP by preventing the formation of toxic bifunctional lesions on DNA. Alternatively, there may be more efficient repair of Pt–DNA adducts formed by the trans isomer, including possible crosslinks between DNA and glutathione. Further studies of these possibilities are warranted.

The repair of specific *cis*-DDP adducts from DNA is also important to evaluate, since repair may play a key role in determining platinum cytotoxicity [69–73,89]. In the best characterized example to date, purified components of the *E. coli* ABC excinuclease repair system efficiently remove *cis*-DDP–DNA adducts in vitro [104,105]. In this system, adducts are excised as dodecanucleotides following endonuclease cleavage of the damaged DNA strand eight nucleotides upstream (5′) and four nucleotides downstream (3′) from the site of platinum binding. The resulting gap is filled in by DNA polymerase and sealed by DNA ligase. Once again, in a

related series of experiments, specific DNA adducts were found not to be processed identically. In particular [Pt(DACH){d(ApG)}] was repaired more rapidly than [Pt(DACH){d(GpG)}], and both were repaired less efficiently than [Pt(DACH){d(GpNpG)}] or monofunctional adducts (where DACH is 1,2-diaminocyclohexane) [105]. The specific DNA distortions responsible for initiating repair are still uncertain. A number of laboratories are currently attempting to isolate the factor(s) responsible for the recognition and subsequent repair of specific *cis*-DDP–DNA adducts in eukaryotic cells. This objective has not been accomplished to date, although proteins that bind specifically to DNA modified by *cis*-DDP have been detected in cell extracts and cloned [106,107].

Another question of scientific and clinical importance is how cells become resistant to *cis*-DDP [108]. It is likely that several different mechanisms contribute, for different studies have already reported increased levels of metallothionein [109,110], glutathione [111,112], thymidylate synthase activity [113] and DNA repair [74–76] in *cis*-DDP-resistant cells. In the first two instances, the cell apparently uses the affinity of platinum for sulfur donor ligands to bind and remove a toxic agent from the cytoplasm. Increased drug efflux, a major resistance mechanism for other chemotherapeutic compounds [114], has not yet been identified in *cis*-DDP-resistant cells. As with DNA repair, an understanding of the mechanisms of *cis*-DDP resistance may help coordination chemists to design complexes that can overcome this problem, which often limits the clinical efficacy of the drug.

Although *cis*-DDP inhibits DNA replication, thereby blocking cell division, the exact mechanisms by which cisplatin actually kills the cell remain obscure. In addition to inhibiting replication, *cis*-DDP also inhibits DNA transcription [102] and amino acid transport [115], although relationships between these activities and cytotoxicity are still unclear. As might be expected, the biochemical basis for the selective cytotoxicity of *cis*-DDP towards certain types of cancer cells is also unknown, since the underlying cause of platinum-induced cell death is still under investigation [116]. It seems reasonable to expect, however, that research into the mechanisms of *cis*-DDP repair, resistance and cytotoxicity will, eventually, also help to explain how cisplatin chemotherapy can cure testicular cancer at dose levels that do little harm to normal cells.

In conclusion, there is currently much detailed information available about the coordination chemistry of platinum anticancer drugs and their analogs with DNA. It now remains to evaluate the persistence and repair of individual adducts in normal and tumor cells from a variety of tissues. Perhaps the anticancer properties of cisplatin arise from the inability of tumor cells to repair lesions at a rate sufficient to assure survival, whereas normal cells of the same kind are more efficient at excising platinum

damage. If such differential repair were responsible for the chemotherapeutic properties of cisplatin, coordination chemists would have a rational basis on which to design and synthesize new and better metal-based anticancer drugs.

(v) New platinum drugs

The original structure–function correlations for platinum anticancer drugs indicated that only bifunctional complexes with two leaving groups in cis positions were active [37–39,117]. It has recently been reported, however, that monofunctional complexes of the kind *cis*-$[Pt(NH_3)_2(X)Cl]^+$, where X is a pyrimidine base or substituted pyridine, are active [118]. These "triamine" complexes bind chiefly to purine nucleotides on DNA, forming monofunctional adducts [119] which, unlike those formed by $[Pt(dien)Cl]^+$ and $[Pt(NH_3)_3Cl]^+$, inhibit replication [103]. Unlike their *cis*- and *trans*-DDP analogs, they do not bend DNA [88]. This example is but one of many approaches currently under investigation by coordination chemists in an attempt to find platinum complexes with antitumor activity exceeding that of cisplatin.

D. PLATINUM COMPOUNDS AS DNA INTERCALATORS

(i) The discovery of metallointercalation

The first indication that coordination compounds could bind by intercalation to nucleic acids came during attempts to label the sulfur atom of 4-thioridine (s^4-U) in *E. coli* $tRNA^{Val}$ with $[Pt(terpy)Cl]^+$ [120]. Incubation of the tRNA with $[Pt(terpy)Cl]^+$ induced a shift of the s^4-U absorption band that was not observed for other metal complexes lacking an aromatic heterocyclic ligand. This result was rationalized by proposing that the planar aromatic platinum complex could stack between the bases of tRNA, facilitating platinum coordination at an otherwise inaccessible sulfur atom. Subsequent studies confirmed that $[Pt(terpy)Cl]^+$ and related complexes do indeed bind to duplex RNA and DNA by intercalating between base pairs in the double helix [121–126]. Intercalative binding to DNA, shown schematically in Fig. 5, has important spectroscopic and structural consequences which we now consider in detail.

Intercalation was first proposed by Lerman to explain the strong affinity for DNA of certain heterocyclic aromatic dyes such as the aminoacridines [127]. Intercalators are of interest owing largely to their ability to inhibit nucleic acid synthesis in vivo, leading to their activity as mutagens, antibiotics, antibacterials, trypanocides, schistosomicides and antitumor agents. Examples of intercalators are given in Fig. 6 [128,129]. The binding energy

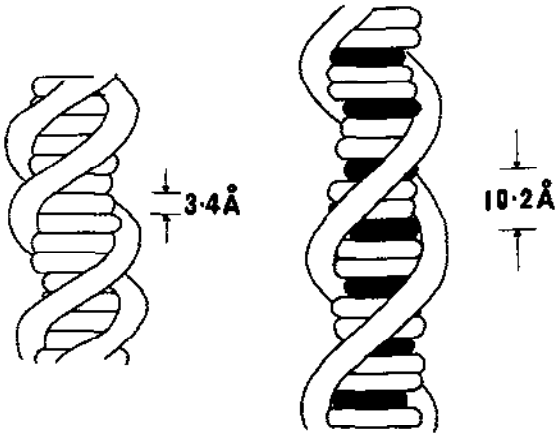
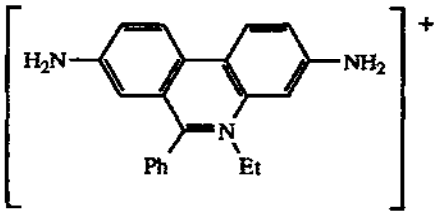


Fig. 5. Schematic representation of double-stranded DNA without (left) and with (shaded area, right) a bound intercalator (reproduced with permission from J.K. Barton and S.J. Lippard, Met. Ions Biol., 1 (1980) 31).

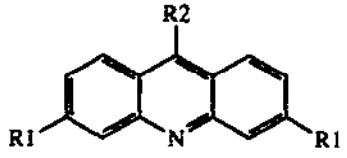
for intercalation derives both from electrostatic and aromatic stacking interactions between the intercalator and DNA, intercalators typically being planar, cationic heterocycles containing two or three fused aromatic rings. These requirements are met by platinum complexes of terpyridine, bipyridine and *o*-phenanthroline in which the platinum coordination plane coincides with that of the aromatic ligand (Fig. 7). In contrast, non-planar platinum complexes such as $[Pt(N1\text{-py})_2(en)]^{2+}$ do not intercalate because the pyridine (py) rings are oriented perpendicular to the platinum coordination plane [130]. Similarly, a "double thick" complex containing two stacked $[Pt(terpy)]^{2+}$ units (Fig. 8) does not intercalate because the double helix cannot accommodate the 6.8 Å width of two stacked aromatic rings [131]. Double intercalation is possible, however, when the two $\{Pt(terpy)\}^{2+}$ units are linked by an α,ω-dithioalkyl chain containing five or more carbon atoms [132,133].

(ii) Properties of metallointercalators

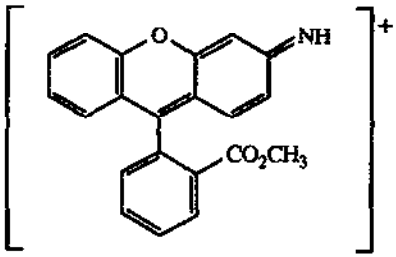
The properties of DNA intercalators, elucidated in studies from many laboratories [134,135], are nicely illustrated by studies of the platinum metallointercalator $[Pt(terpy)(HET)]^{+}$ (Fig. 7). In the absence of DNA, most intercalators self-associate in aqueous solution and stack in the solid state. Favorable stacking of planar d^8 metals is a well-known phenomenon that applies to an extensive class of planar platinum(II) complexes [136]. Such stacking interactions are further enhanced by the presence of aromatic ligands such as terpyridine. It is thus not surprising that the metallointerca-



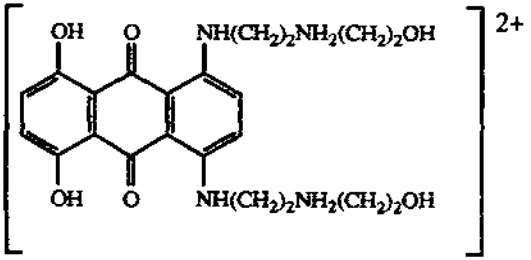
Ethidium



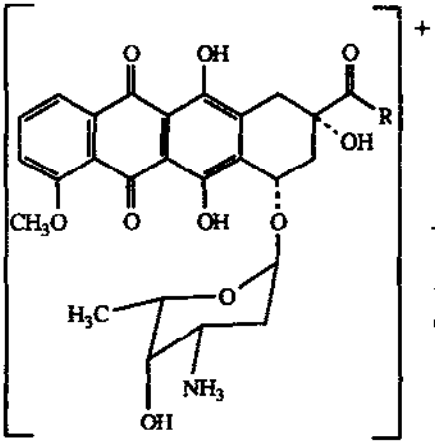
Proflavin; R1 = NH_2, R2 = H
Acridine Orange; R1 = $N(Me)_2$, R2 = H
9-Aminoacridine; R1 = H, R2 = NH_2

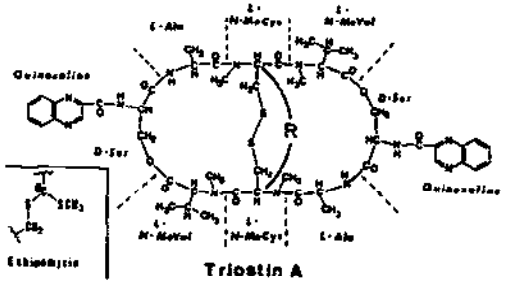


Rhodamine 123



Mitoxatrone





Triostin A

Daunomycin; R = CH_3
Doxorubricin; R = CH_2OH

Triostin A; R = -CH_2-S-S-CH_2-
Echinomysin; R = -$CH(SCH_3)$-S-CH_2-

Fig. 6. Structures of DNA intercalators and dyes. The structures of Triostin A and Echinomysin are reprinted from ref. 155.

lator $[Pt(terpy)(HET)]^+$ dimerizes both in solution ($K_{dimer} = 7(5) \times 10^3$) and in the solid state [123].

Intercalative binding to DNA is fully reversible, with association con-

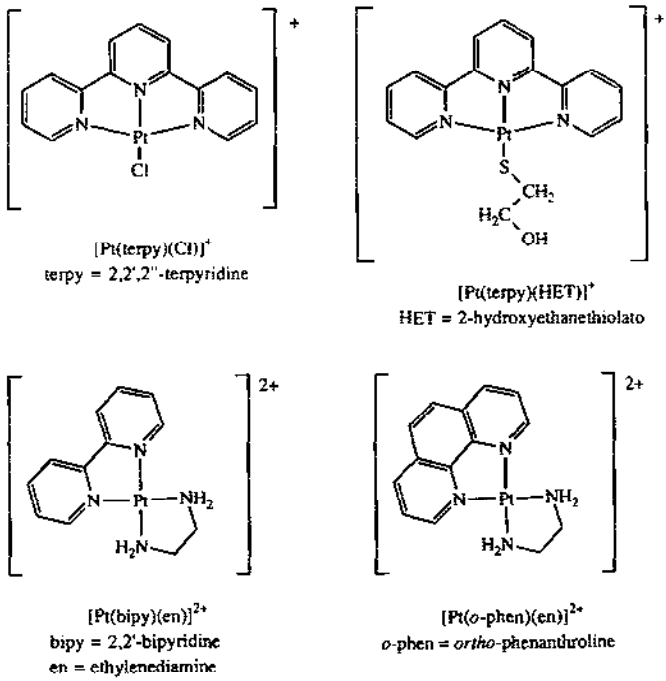


Fig. 7. Structures and abbreviations of platinum intercalators.

stants typically of the order of 10^5–10^6 M^{-1}. The absorption and fluorescence spectra of a compound are usually perturbed on intercalative binding to DNA, and spectroscopy is commonly used to quantitate the interaction [137]. For example, there is a 20-fold enhancement in ethidium fluorescence upon intercalation [138,139]. This fluorescence enhancement is commonly used in the detection of nucleic acids [140,141] and to determine the binding

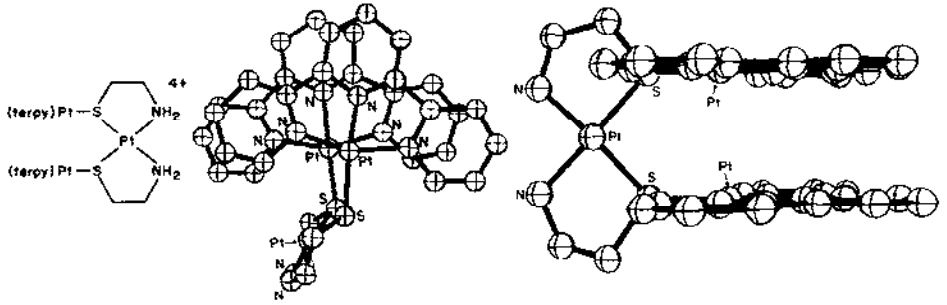


Fig. 8. Schematic structure (left) and ORTEP view (right) of a "double thick" platinum terpyridine complex.

affinity of a different intercalator that competitively inhibits ethidium binding [138]. Both competitive inhibition and direct binding experiments reveal the DNA affinity of $[Pt(terpy)(HET)]^+$ to be about $10^5\ M^{-1}$. Detailed studies have revealed that $[Pt(terpy)(HET)]^+$ binds preferentially at sites adjacent to at least one G – C base pair. Moreover, intercalation at one site excludes the binding of a second molecule between the immediately adjacent base pairs, according to the nearest-neighbor exclusion principle [125]. Binding of $[Pt(terpy)(HET)]^+$ to double-helical RNA is even more complex, since the intercalator binds cooperatively and can exceed the nearest-neighbor exclusion limit [126]. Such complexity is typical of many intercalators and reflects the site heterogeneity of the DNA receptor and the complex equilibria associated with intercalative binding [137,142–146].

The bulk physical properties of DNA also change on intercalator binding since the insertion of a planar chromophore stabilizes, lengthens, stiffens and unwinds the double helix [127,142,147,148]. These principles are again illustrated by the intercalator $[Pt(terpy)(HET)]^+$, which raises the melting temperature and specific viscosity of DNA on binding. The platinum complex also unwinds DNA to nearly the same extent as ethidium, a value experimentally determined to be 26° per average DNA binding site [149]. Molecular details of intercalative binding have emerged with the cocrystallization of various intercalators bound to small synthetic DNA and RNA oligonucleotides [150,151]. Most X-ray structure determinations to date have been carried out on complexes of simple intercalators bound to dinucleotides, although hexanucleotide or octanucleotide complexes with daunomycin [152] and the bis-intercalators triostin A [153,154] and echinomysin [155] have also been solved.

(iii) Structural details

The first crystal structure of a metallointercalator bound to a deoxynucleotide fragment was the 2 : 2 complex of $d(CpG)^-$ and $[Pt(terpy)(HET)]^+$ [156]. In this complex, one $[Pt(terpy)(HET)]^+$ cation intercalates within a Watson–Crick base-paired $[d(CpG)]_2^{2-}$ duplex while the second stacks between $[d(CpG)_2]^{2-}$ units to form an infinite column that is electrically neutral (Fig. 9). The structure reveals that $[Pt(terpy)(HET)]^+$ intercalates symmetrically between base pairs with the hydroxyethanethiolato chain projecting into the major groove of the miniature DNA helix. The O6 atoms of the guanine rings are located almost directly above and below the intercalated platinum atom (3.4 Å) and may enhance the G–C specificity of the intercalator [132,156], although preferential intercalation at G–C sites is a general phenomenon that can also be explained by the greater polarizability of this base pair [142,157].

As previously noted, intercalation lengthens and unwinds the DNA

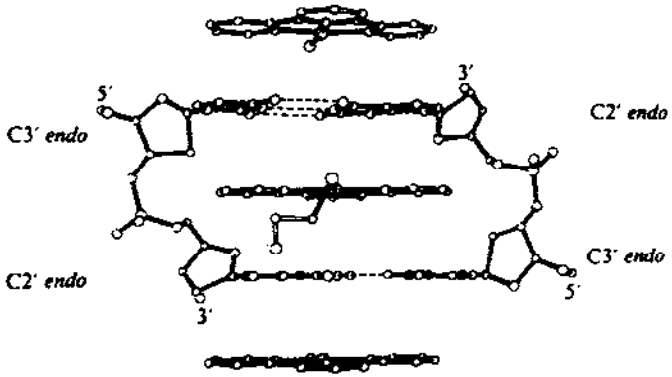


Fig. 9. Structure of the 2:2 complex between $d(CpG)^-$ and the platinum intercalator $[Pt(terpy)(HET)]^+$ (reproduced with permission from ref. 156).

double helix. Lengthening is directly observed in the $[(terpy)Pt(HET)]_2 \cdot [d(CpG)]_2$ structure where the G–C base pairs are separated by 3.4 Å, to a distance of 6.8 Å, in order to accommodate the intercalator. The right-handed dinucleotide helix in this structure is also underwound by approximately 21° relative to the usual winding angle of 34° per base pair in an idealized B-DNA structure [158]. An additional feature of interest in the intercalation complex is a change in sugar pucker of the 5′ dC base from the C2′-*endo* conformation normally observed in B-DNA to a C3′-*endo* conformation. The 3′-deoxyguanosine sugar, however, shows a C2′-*endo* pucker typical of B-DNA. This pattern of C3′-*endo*-(3′-5′)-C2′-*endo* alternating sugar pucker has been observed in many but not all DNA intercalator complexes and further illustrates the conformational flexibility of DNA. In spite of the difficulties in generalizing from a complex as small as a dinucleotide, the $[Pt(terpy)(HET)]_2 \cdot [d(CpG)]_2$ structure, taken together with the structures of other similar intercalation complexes, clearly confirms the intercalative binding mode originally assigned [121] for $[Pt(terpy)(HET)]^+$ and offers a structural basis for the unwinding and lengthening [124,125] of DNA by the intercalator.

(iv) Proof of the neighbor exclusion principle

Binding of platinum-based intercalators to full length DNA has been investigated in fiber diffraction studies which exploit the high electron density of these complexes [130,159,160]. In particular, these studies have addressed the structure of DNA at saturation binding levels of the intercalator in order to understand the structural basis of nearest-neighbor exclusion. Diffraction patterns from oriented DNA fibers with intercalated

$[Pt(terpy)(HET)]^+$ revealed an intense, near-meridional layer line with a spacing of 10.2 Å, not present in control DNA fibers [159]. This 10.2 Å spacing strongly supports a neighbor-excluded model with a repeating structural unit comprised of an intercalator stacked between two base pairs. These units can be further stacked to give a polymer with a repeating structure (10.2 Å) in which intercalators are excluded from every other binding site (Fig. 5). Further analysis of the diffraction pattern suggested that the bases and intercalators stack in an untwisted column, rather than in a helix as originally supposed [160]. A structure in which the nucleotides have alternating C3′-*endo,syn* and C2′-*endo,anti* conformations was assigned on stereochemical grounds. Such an arrangement provides two distinct intercalator binding sites along the polymer, thus rationalizing the exclusion of intercalators from every other site. This intercalator-stabilized, unwound DNA duplex is a striking example of the polymorphic nature of DNA. It also illustrates the value of metallointercalators as a class of heavy atom probes that afford structural information not available using organic analogs such as ethidium bromide [159].

E. LINKED Pt–INTERCALATOR COMPLEXES

(i) Tethered molecules

A recent development has been the design and synthesis of complexes in which an intercalator and a chemically reactive platinum functionality are covalently linked (Fig. 10). The interactions of these and other multifunctional molecules with nucleic acids are of interest since they introduce potentially new modes of binding and can yield information on the mutual interactions of functional groups held in close proximity. A longer term goal of this work is to develop complexes that will bind DNA in predetermined ways and that can modify specific DNA functions such as replication and transcription. The first such Pt–intercalator complex to be synthesized was $[Pt\{AO(CH_2)_6en\}Cl_2]^+$, a cation in which a hexamethylene chain links the reactive platinum moiety $\{Pt(en)Cl_2\}$ to the DNA intercalator acridine orange (AO) [161–163]. The structure of the oxalato (ox) analog, $[Pt\{AO(CH_2)_6en\}(ox)]^+$, is shown in Fig. 11. The $[Pt\{AO(CH_2)_6en\}Cl_2]^+$ cation binds covalently to DNA through its $\{Pt(en)\}^{2+}$ moiety while the AO ring intercalates one or two base pairs away. This multifunctional mode of binding unwinds DNA to an extent greater than observed for the binding of either component alone. This complex has been used to probe mutual interactions between platinum antitumor drugs and DNA intercalators, as described in Section F.

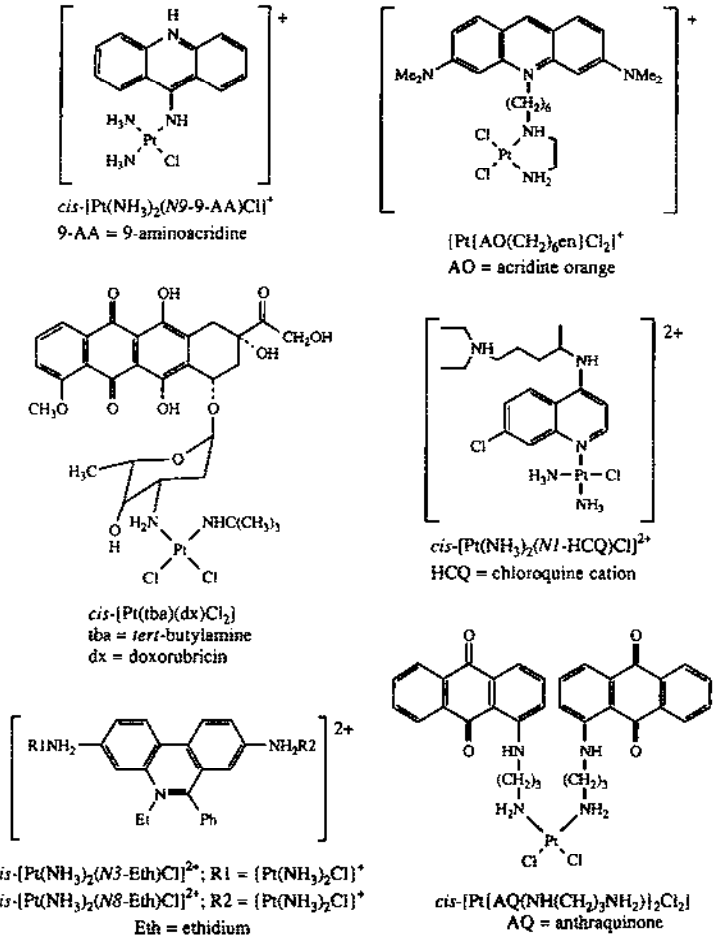


Fig. 10. Structures and abbreviations of platinum complexes covalently linked to DNA intercalators.

(ii) Directly linked complexes

A second type of complex having the potential to bind DNA both covalently and intercalatively contains a reactive platinum moiety bound directly to an intercalator. Intercalator-bound analogs of both *cis*-DDP and the biologically active "triamine" complexes [118] have been synthesized. One such complex (Fig. 12) is *cis*-$[Pt(NH_3)_2(N9\text{-}9\text{-}AA)Cl]^+$, in which platinum is bound to the N9 position of the imino tautomer of 9-aminoacridine (9-AA) [164]. This complex is structurally interesting because the rigid geometry of coordinated 9-AA forces the apical H1 proton to lie within 2.4 Å of the platinum center. As predicted both theoretically [165]

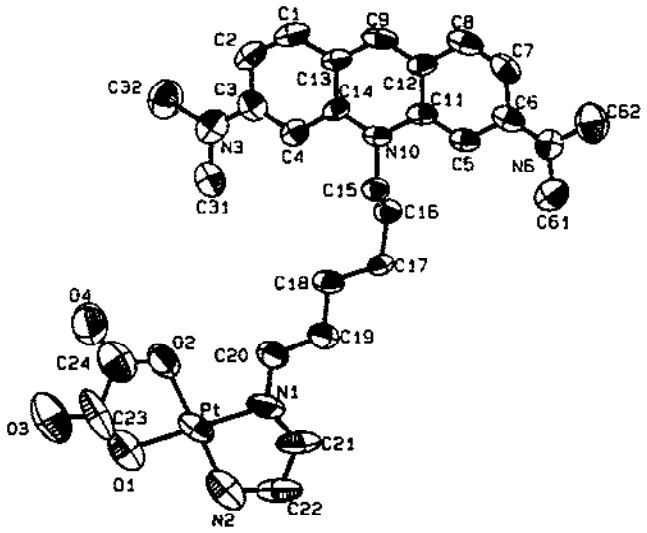


Fig. 11. Structure of the $[Pt\{AO(CH_2)_6en\}(ox)]^+$ cation.

and experimentally [166], this proton is deshielded by approximately 3.3 ppm owing to the paramagnetic anisotropy of the platinum atom. There is, however, no evidence for agostic interactions [167] in this or related complexes [164]. Another directly bound Pt–intercalator complex is *cis*-$[Pt(tba)(dx)Cl_2]$, in which the ammonia ligands of *cis*-DDP have been replaced by *tert*-butylamine (tba) and the intercalator doxorubicin (dx), the latter coordinating through its N3′ amino group [168,169]. Although both complexes contain intercalating and reactive platinum functionalities, it may be stereochemically difficult for the doxorubicin and 9-aminoacridine com-

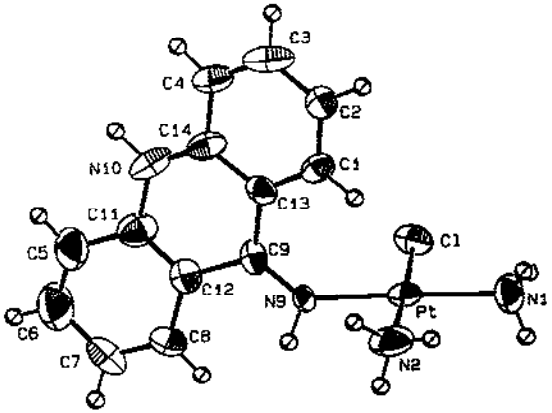


Fig. 12. Structure of the *cis*-$[Pt(NH_3)_2(N9\text{-}9\text{-}AA)Cl]^+$ cation.

plexes simultaneously to intercalate and bind covalently at purine N7 positions in the major groove of DNA [152,170]. This expectation has been confirmed for *cis*-[Pt(tba)(dx)Cl_2], which binds intercalatively, but not covalently, to DNA in vitro [169]. Covalent binding to DNA may also be kinetically difficult for *cis*-[Pt$(NH_3)_2$(*N9*-9-AA)Cl]$^+$ since 9-AA normally intercalates with its exocyclic N9 position oriented towards the minor groove [170].

Complexes that may have the correct stereochemistry for both intercalative and covalent platinum binding to DNA have been synthesized using either chloroquine (CQ) [164] or ethidium (Eth) [171] as ligands (Fig. 10). In the complex *cis*-[Pt$(NH_3)_2$(*N1*-HCQ)Cl]$^{2+}$, platinum is bound to the N1 endocyclic amino group of the chloroquine cation (HCQ), a position thought to reside in the major groove of DNA following intercalation [172,173]. Subsequent reaction of platinum with the N7 atom of an adjacent purine nucleobase should therefore be facilitated, although the DNA binding of *cis*-[Pt$(NH_3)_2$(*N1*-HCQ)Cl]$^{2+}$ has not yet been investigated. The complexes *cis*-[Pt$(NH_3)_2$(*N3*-Eth)Cl]$^{2+}$ and *cis*-[Pt$(NH_3)_2$(*N*8-Eth)Cl]$^{2+}$, in which platinum is coordinated to an exocyclic amino group of ethidium, have also been synthesized. The relatively weak arylamine–Pt bonds in these complexes slowly hydrolyze in aqueous solution. Moreover, the more acidic N3 coordination isomer may be reversibly deprotonated (pK_a N3 = 9.8) resulting in a color change from yellow to deep blue [174]. DNA binding and modeling studies described below indicate that both isomers can react with DNA covalently and intercalatively. Platinum has also been reported to form covalent or ionic compounds with other dyes and intercalators including acridine orange [175], proflavin [175], mitoxantrone [176], rhodamine [42,177] and anthraquinone [178]. Although the toxicity of many Pt–intercalator complexes precludes their use as drugs, antitumor activity has been reported for [Pt{AO$(CH_2)_6$en}Cl_2]$^+$ [173], *cis*-[Pt(tba)(*N3'*-dx)Cl_2] [168] and for platinum complexes of mitoxantrone [176].

F. INTERACTIONS OF *cis*-DDP AND INTERCALATORS ON DNA

The binding of *cis*-DDP to DNA in the presence of intercalators has been studied in an effort to understand the synergism often observed between these two classes of antitumor drugs. Such studies also provide insight into how the local tertiary structure of DNA, which can be modulated by intercalators, might affect the site specificity of *cis*-DDP binding [179–181]. Early footprinting studies indicated that ethidium bromide alters the nuclease-sensitive platinum binding sites on DNA [179]. In particular, a d(G_6CG_2) sequence in a 165 base-pair DNA restriction fragment, not detectably modified by *cis*-DDP in the absence of ethidium, bound platinum

in a dose-dependent fashion if the DNA was pretreated with ethidium bromide. It was suggested that ethidium might facilitate *cis*-DDP binding by modifying the local DNA structure. Alternatively, the intercalator could effect DNA binding by changing the mode of DNA coordination from bidentate to monodentate. Subsequent work demonstrated the validity of the latter explanation and the likelihood of the former. It should be noted that interactions occurring between intercalators and platinum complexes at relatively high levels of DNA modification (drug-to-nucleotide ratio, or D/N, $= 10^{-1}$–10^{-3}) may be less important at the much lower levels of DNA modification by platinum in biological systems (D/N $= 10^{-5}$–10^{-6}).

The first evidence that ethidium could change the mode of *cis*-DDP coordination to DNA came from EM studies in which the pronounced shortening of circular DNA molecules on platinum binding was found to be inhibited when platination was carried out in the presence of ethidium. Subsequent work revealed formation of a "ternary" complex between *cis*-DDP, ethidium and DNA [182–184]. In this complex, ethidium could not be removed by butanol extraction, filtration at acidic pH, or TLC separation at basic pH, conditions that quantitatively remove intercalatively-bound ethidium. A covalent link between platinum and ethidium was suggested by the blue shift in the absorption spectrum and quenching of the fluorescence of the ternary complex compared with intercalated ethidium. The chemical nature of the ternary complex was uncertain, however, since it slowly ($t_{1/2} \approx 10$ h) lost ethidium on standing at 37 °C. Furthermore, in the absence of DNA, *cis*-DDP and ethidium did not react to any appreciable extent under conditions used to form the ternary complex [180,182,183].

The composition of the ternary complex was revealed by first preparing and characterizing the precursor complexes *cis*-$[Pt(NH_3)_2(N3\text{-Eth})Cl]^{2+}$ and *cis*-$[Pt(NH_3)_2(N8\text{-Eth})Cl]^{2+}$ and subsequently demonstrating their reactions with DNA to form adducts spectroscopically identical with those obtained in the DNA-promoted reaction of *cis*-DDP with ethidium [171]. The ternary complexes must therefore consist of platinum coordinated by two cis ammines, an N3- or N8-bound ethidium and a DNA ligand, most likely the N7 atom of either guanine or adenine. A remarkable feature in the formation of the Pt–Eth–DNA complex is that DNA actually promotes the reaction between *cis*-DDP and ethidium. Although ethidium does coordinate to *cis*-DDP in the absence of DNA, the reaction proceeds to the same extent at 60-fold lower concentrations of both reactants when carried out in the presence of DNA. There are several related examples in which DNA directs the diffusion of macromolecules [185–187] or modulates the reactions of small molecules [188–190], the molecular details of which are of considerable interest. A structural model (Fig. 13), based on known modes of *cis*-DDP and ethidium binding to DNA, has been proposed that nicely

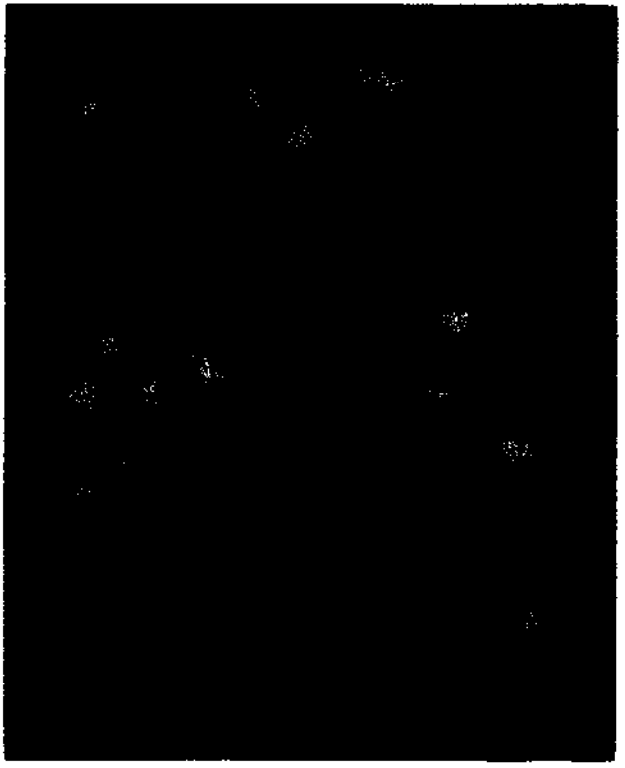


Fig. 13. Proposed intermediate in the DNA-promoted reaction of *cis*-DDP with ethidium. This model is comprised of a duplex DNA hexamer, d[C(1)pG(2)pC(3)pG(4)pC(5)pG(6)]·d[C(7)pG(8)pC(9)pG(10)pC(11)pG(12)], in which ethidium is intercalated between the C(3)·G(10) and G(4)·C(9) base pairs, and an idealized *cis*-$\{Pt(NH_3)_2Cl\}^+$ fragment is coordinated to N7 of G4 (see ref. 171 for full discussion).

accounts for the facile reaction of ethidium with platinum following monofunctional binding adjacent to intercalated ethidium. In this model, DNA acts as a template to position the ethidium amino groups above the platinum coordination plane in the major groove of the double helix, effecting nucleophilic displacement of the second chloride ligand.

The role of DNA as a template in this reaction is supported by the observation that double-stranded DNA is absolutely required and by the preferential reactivity of the heterocopolymers poly(dG–dC) · poly(dG–dC) and poly(dA–dC) · poly(dG–dT) for which both ethidium intercalation and monofunctional coordination of *cis*-DDP are favored [182,183]. The reaction also depends on the stereochemistry of the intercalator and the platinum complex, observations readily interpreted in the light of the proposed structural model. Thus ethidium modulates *cis*-DDP binding to DNA by

forming a ternary Pt–Eth–DNA complex. A reasonable structural model has been proposed to explain how DNA serves as a template to promote this reaction.

Intercalators can also influence *cis*-DDP binding by altering the structure of DNA. Platinum binding is dramatically affected by changes in DNA tertiary structure; for example, *cis*-DDP forms different adducts with the B and Z forms of poly(dG–m^5dC) · poly(dG–m^5dC) [191]. Moreover, the B → A and B → Z DNA transitions are inhibited by bound *cis*-DDP [192–194]. These results suggest that *cis*-DDP binding should be sensitive to sequence-dependent local DNA structure modulations such as those accompanying intercalator binding. As noted above, such appears to be the case for one d(G_6CG_2) sequence in a 165 base-pair restriction fragment and may be true for d(CGG) sequences in general [162,195]. *cis*-DDP binding at many such sequences is enhanced in the presence of ethidium. Although the stereochemical rules for platinum coordination at local DNA conformations are not yet understood, the emerging wealth of DNA structural information offers several interesting possibilities. For example, A-like d(CGG) sequences in several oligonucleotide crystal structures show interstrand guanine–guanine stacking interactions at the d(CpG) step that might disfavor *cis*-DDP coordination compared with the more typical intrastrand stacking interactions in B-DNA double helices [196,197]. Ethidium binding within such a sequence might open up the structure to promote *cis*-DDP binding, although the molecular details of such a process are not yet understood.

Other intercalators, including proflavin (Fig. 6) which also forms ternary complexes with *cis*-DDP, do not enhance platinum binding at excluded sites. Mapping studies and measurements of intercalator residence times on DNA suggest that local changes in DNA structure caused by intercalation must persist long enough for *cis*-DDP to diffuse to the site if enhanced binding is to occur [162,195]. This hypothesis is strengthened by studies with acridine orange and with the platinum-linked acridine orange complex [Pt{AO $(CH_2)_6$en}Cl_2]$^+$. Acridine orange does not alter enzyme detectable platinum binding sites on DNA unless covalently linked to platinum by the hexamethylene chain. This result suggests that the lifetime of an AO-modified DNA duplex may be too short to enhance platinum binding unless the platinum moiety is held at a high local concentration by the linker chain.

G. SUMMARY AND A LOOK TOWARDS THE FUTURE

Since the discovery of the antineoplastic activity of *cis*-DDP, considerable progress has been made in understanding how platinum complexes bind to DNA. In addition, several new compounds have been synthesized that hold

considerable promise as heavy atom probes for nucleic acid structure. The most detailed advances have been in understanding how *cis*-DDP binds to DNA. The DNA adduct profile is known and numerous studies have yielded a reasonably complete picture of the structures of *cis*-DDP–DNA adducts. There is still a need, however, for the high resolution structural information that could be provided by crystal structures of the major *cis*-DDP adducts within double-stranded oligonucleotides. Questions also remain about how local DNA structure affects the binding of *cis*-DDP. Nevertheless, many of the most important remaining questions now focus on the biological consequences of platinum binding to DNA.

As has been emphasized, knowledge of how platinum antitumor drugs work would have major implications for the further design and improvement of these inorganic drugs. An increased understanding of DNA structure and DNA–drug interactions means that, in a simple sense, it is becoming possible to design platinum complexes to bind DNA in predictable ways. Examples of such coordination compounds include Pt–intercalator complexes, bis-intercalator complexes and multimeric *cis*-DDP analogs [198]. An important caveat, however, even for simple DNA binding, is the complexity introduced by DNA polymorphism. Local sequences affect local structures in a manner that can determine not only what platinum adducts form but whether they form at all. Given the many additional variables affecting therapeutic activity, it is not yet reasonable to expect to design better platinum antitumor complexes in a completely rational way. We still have limited understanding of the structural features necessary to disrupt DNA function, minimize repair or selectively target tumor cells. Furthermore, the fact remains that, despite the synthesis and testing of literally thousands of derivatives in the past 20 years, *cis*-DDP remains the most effective platinum antitumor drug. Nevertheless, studying the reactions of platinum complexes with DNA contributes to our knowledge of basic platinum coordination chemistry, provides fundamental information about DNA structure and small molecule interactions, and may, as our understanding of drug mechanisms improves, lead to the synthesis of more effective drugs. Such research endeavors also have the potential to uncover important information about biological events connected with cancer. For, to make effective progress in uncovering the molecular mechanism of cisplatin, the coordination chemist must learn and apply the tools of the molecular and cell biologist. In so doing, his or her path will ultimately cross that of biochemists who study cellular events that cause cancer. Exciting chapters in the bioinorganic coordination chemistry of platinum and other metal-based antitumor drugs surely lie ahead.

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