

THE CHEMICAL AND BIOCHEMICAL CONSEQUENCES OF THE BINDING OF THE ANTITUMOUR DRUG CISPLATIN AND OTHER PLATINUM GROUP METAL COMPLEXES TO DNA *

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A. INTRODUCTION

Although the six members of the platinum group, ruthenium, rhodium, palladium, osmium, iridium and platinum, exhibit distinctive properties and their chemistry has some common features, there are nevertheless variations, depending on oxidation state, stereochemistry etc. Platinum and palladium have attracted continual attention largely because they have been the source of new compounds of high intrinsic interest, particularly with respect to bonding and structure. Platinum(II), rhodium(I), iridium(I) and palladium(II) undergo oxidative addition reactions and molecules such as

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Cl_2 , HCl etc. can add across the plane. Platinum(II) complexes can be oxidized to platinum(IV) with retention of configuration (*cis* and *trans*). The primary medical use to-day of platinum group metals is in cancer chemotherapy.

The interactions of metal ions with biomolecules and the functions of metal ions in physiological systems are very complex, and the precise nature of these interactions or processes is for the most part unknown. The discovery by Rosenberg et al. [1,2] that some platinum complexes, particularly *cis*-diamminedichloroplatinum(II) (*cis*-DDP or cisplatin) (Fig. 1) exhibit carcinostatic properties (not shared by the *trans* isomer) has given considerable impetus to research in the area of metal ion interactions with nucleic acid constituents. Cisplatin is a clinically important antitumour drug currently used in hospitals all over the world in the treatment of advanced metastatic, testicular and ovarian cancers. High overall response rates were reported for the drug when used alone or in combination with vinblastine, bleomycin or doxorubicin. Many recent clinical advances suggest that it will be also of significant utility in the treatment of other solid cancers such as those of the bladder, prostate, lung, head and neck, certain cancers in children and in other genito-urinary cancers. However, the drug is highly toxic. The major toxicities induced by cisplatin include nephrotoxicity, nausea and vomiting, myelosuppression and ototoxicity. Other toxicities include anaphylactic-like reactions, neurotoxicity characterized primarily by peripheral neuropathies, hypocalcemia secondary to renal damage and occasionally cardiac abnormalities, elevation of liver enzymes and allergic reactions. All toxicities appear to be dose related and nephrotoxicity, ototoxicity and neurotoxicity appear to be cumulative [3].

Almost from the initial discovery of cisplatin's antitumour activity, attempts have been made to achieve its therapeutic benefits while lowering its toxicity. These consisted of altering treatment methods by administering large volumes of fluids or combinations of other drugs or radiotherapy with cisplatin, in addition to modifying the drug itself by varying the metal ion and its attached ligands [4]. As the need to develop a less toxic drug with greater therapeutic properties than cisplatin became evident, efforts were made on a world-wide basis to synthesize new platinum compounds possessing these characteristics. Second generation drugs have clearly emerged with therapeutic activity comparable to that of cisplatin and associated with considerably reduced toxicity. Of these, carboplatin (*cis*-diammine(1,1-cyclobutanedicarboxylato)platinum(II)) (Fig. 2) is the most advanced and has been launched commercially in the U.K. This drug was approved for the treatment of ovarian cancer and for small cell lung cancer. Phase II/III studies [5] for the treatment of ovarian cancers at the Royal Marsden Hospital, London, showed activity equivalent to that of cisplatin with a

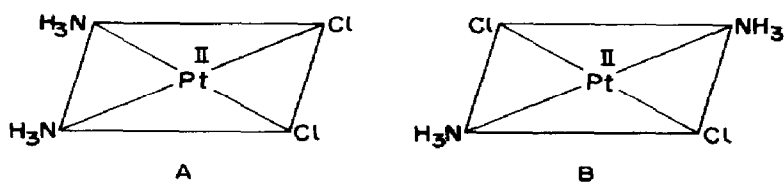
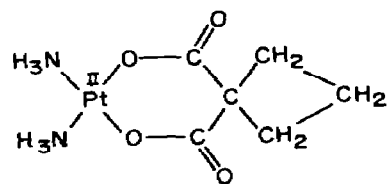
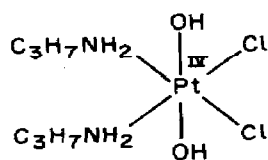


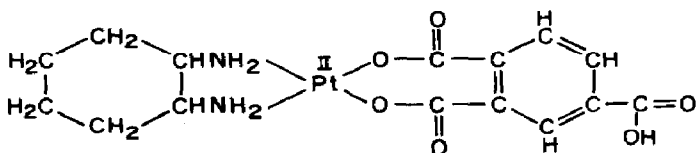
Fig. 1. Structure of (A) *cis*-diamminedichloroplatinum(II) and (B) *trans*-diamminedichloroplatinum(II).



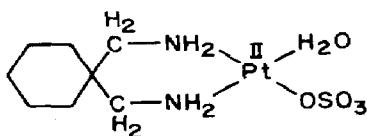
Carboplatin (*cis*-diammine(1,1-cyclobutanedicarboxylato)platinum(II))



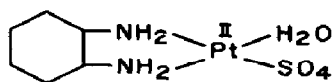
Iproplatin (*cis*-dichloro-*trans*-dihydroxybis(isopropylamine)platinum(IV))



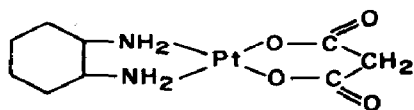
DACCP (4'-carboxyphthalato(1,2-diaminocyclohexane)platinum(II))



Spiroplatin (aquo-1,1-bis(aminomethyl)cyclohexanesulphatoplatinum(II))



Sulphato(1,2-diaminocyclohexane)platinum(II)



Malonato(1,2-diaminocyclohexane)platinum(II)

Fig. 2. Structures of clinically important antitumour platinum complexes.

similar long-term survival rate of about 30% but producing markedly less toxicity. Other toxicities normally associated with cisplatin (kidney toxicity, peripheral nerve damage, hearing loss) were almost totally absent. Clinical interest in other platinum analogues (Fig. 2) remains high [6]; these are already undergoing trials and may soon be marketed. The list includes iproplatin (*cis*-dichloro-*trans*-dihydroxybis(isopropylamine)platinum(IV)), DACCp (4'-carboxyphthalato(1,2-diaminocyclohexane)platinum(II)), spiroplatin (aquo-1,1-bis(aminomethyl)cyclohexanesulphatoplatinum(II)) [7], sulphato(1,2-diaminocyclohexane)platinum(II) [8], malonato(*trans*-($-$)1,2-diaminocyclohexane)platinum(II), ethylenediamine(malonato)platinum(II) [9] and *cis*-1,1-cyclobutanedicarboxylato(2*R*)-2-methyl-1,4-butanediamine-platinum(II) [10]. At the same time, however, the search continues for third generation platinum complexes, with higher or improved antitumour activity and with reduced toxicity, particularly renal and gastric, which are most troublesome.

X-ray structural analysis of carboplatin (*cis*-diammine(1,1-cyclobutanedicarboxylato)platinum(II)) shows that the coordinated dicarboxylate ligand is not flat, but rather is puckered, having the cyclobutane moiety significantly displaced above the donor plane of the compound [11,12]. Conductance measurements on aqueous solutions of the compound demonstrated that unlike cisplatin, the complex resists aquation [13]. Although this observation is probably due to the fact that the dicarboxylate is a bidentate ligand and thus is more difficult to displace from the platinum ion than a monodentate ligand, e.g. Cl^- , steric factors associated with the puckered dicarboxylate ring may also play a role in substitution reactions with the compound. Since the cyclobutane moiety of the dicarboxylate ligand is displaced significantly above the coordination plane and, as is evident from NMR studies, is oscillating between both sides of the plane [11], its presence may partially block substituting ligands from approaching the platinum ion. This type of steric effect would explain the observed slow rates of binding of carboplatin and the related hydroxymalonato complex to components present in human plasma [14]. If steric factors are important, a complex containing the unsubstituted malonate ligand would be expected to react more quickly with plasma components than either carboplatin or the dihydroxymalonato complex. The reduced ability of carboplatin to undergo substitution reactions is also evident from studies with L1210 cells [15], wherein it was shown that the peak levels of DNA-protein and DNA-inter-strand cross-links induced by carboplatin were found to occur 6–12 h after those produced by cisplatin. The X-ray structural analysis [16] of a platinum complex containing an unsubstituted malonato ligand, *cis*-diammine-(malonato)platinum(II), shows that the coordinated malonato ligand and the platinum atom are in the same plane with the CH_2 group 0.9 Å away from

this plane. This complex may react more quickly with the plasma components than the substituted dihydroxymalonato complex or carboplatin.

The pharmacology of carboplatin appears to be strongly influenced by the slower substitution kinetics of the compound than of cisplatin. Tests performed *in vitro* have shown that it is considerably more stable in plasma at 37°C than cisplatin with half-lives of 30 h and 1.5–3.6 h respectively [17]. The drug is mainly excreted through the kidney, and unlike cisplatin, is recovered largely unchanged. In all animal species studied so far, carboplatin appears to be more rapidly and completely cleared from the tissue and organs than cisplatin after administration of doses of comparable potency. However, it does not appear to be active in cancers of the upper gastrointestinal tract, in breast cancer [18] or on non-small cell lung cancer [19].

The platinum in iproplatin, *cis,cis,trans*-[Pt(NH₂-ⁱPr)₂Cl₂(OH)₂], another likely second generation drug, is in oxidation state IV. Although platinum(IV) compounds can be reduced readily, their substitution reactions are normally very slow unless catalysed. Analysis of the urine and plasma of cancer patients receiving iproplatin identified significant amounts of the anti-tumour-active reduction product of the compound, namely, *cis*-dichlorobis-(isopropylamine)platinum(II). The presence of the divalent complex strongly suggested that iproplatin was deriving its antitumour effects via *in vivo* reduction to the platinum(II) complex or other biologically active platinum(II) compounds. *In vitro* studies with iproplatin showed that, although the compound does not bind to components in human plasma [14], it probably utilizes DNA as a biological target site. It was reported that the compound was capable of cleaving the closed circular form of PM2-DNA [20–22]. X-ray structural analysis [23,24] of iproplatin and the related complex, *cis,cis,trans*-[Pt(IV)(NH₃)₂Cl₂(OH)₂] (oxoplatin), revealed that both compounds formed stable perhydrate complexes with hydrogen peroxide, the reagent used in the synthesis of platinum(IV) complexes, and both perhydrate and non-perhydrate forms of complexes were incapable of DNA strand scission. Lattice hydrogen peroxide appeared to be the agent responsible for the strand breakage in the earlier experiments involving iproplatin. In subsequent experiments pertaining to mechanism, it was shown that biological reducing agents such as iron(II) and ascorbate could reduce iproplatin and oxoplatin to their divalent counterparts, dichlorobis-(isopropylamine)platinum(II) and cisplatin respectively, and that as expected, both reduction products can bind to and unwind supercoiled DNA [25,26]. The prospect that DNA itself or its components may also be capable of carrying out the reduction to platinum(II) has recently been uncovered. Studies have shown that incubation of the platinum(IV) compound, iproplatin, with mononucleotide, 5'GMP, results in a platinum(II) product containing two coordinated mononucleotides [27]. It has also been reported

that incubation of calf thymus DNA with iproplatin and oxoplatin for extended periods of time (12–14 days) results in platination of DNA [28]. Although binding without reduction may in fact be occurring, the oxidation state of platinum bound to DNA was not established in the later study.

Compared with cisplatin ($cis\text{-[Pt(NH}_3)_2\text{Cl}_2]$), iproplatin reacts very slowly with nucleotides. In order to be active, iproplatin must be modified to a platinum(II) form and is in fact reduced by iron(II) or by ascorbic acid [25]. The reduced diaquo form of iproplatin, $cis\text{-[Pt(NH}_2^i\text{Pr)}_2(\text{OH}_2)_2]^{2+}$, has an unusual property in that the second step of its reaction with 5'-guanosine monophosphoric acid (5'GMP) has a positive entropy of activation. In this reaction, there are three bulky groups, i.e. 5'GMP and two NH_2Pr^i ligands, around the platinum. This fact, together with the positive entropy have led Evans and Green [29] to suggest that in this reaction there is a dissociative pathway. Most platinum(II) substitution reactions involving platinum(II) have negative entropies of activation and proceed by an associative pathway [30].

B. CHEMICAL STUDIES

(i) *Reactions of cisplatin in aqueous media*

The encouraging and rapid progress in clinical development of anti-tumour platinum drugs has been the main impetus behind studies of the mechanism of their action. Currently [31] the most convincing mechanism for the cytotoxic action of these agents on cells in culture is that reactions with DNA impair its function as a template for further DNA replication. The anticancer action of $cis\text{-[Pt(NH}_3)_2\text{Cl}_2]$ is thought to involve coordination to a guanosine residue in DNA. However, little is at present known about the specific covalent attachment sites of cisplatin and platinum–uracil blues on DNA. Various studies have implicated N(7) and possibly O(6) of guanine [32–35].

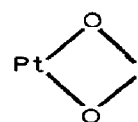
It has long been a matter of interest to what extent cisplatin ($cis\text{-DDP}$) itself or some of its hydrolysis products are responsible for particular biological effects in vivo. In aqueous solution, both chloride ions are slowly lost from the coordination sphere of the platinum(II) ion and water or hydroxide ion becomes bound. Thus a distribution of species is set up, involving the presence of unhydrolysed and partially hydrolysed species. This equilibrium is, however, labile, and under certain conditions of pH and metal ion concentration, hydroxy-bridged polymeric species can be formed. Both dimeric and trimeric species have been isolated from solution and their structures have been determined by X-ray crystallography [36]. Rosenberg [37] reported preliminary studies of the NMR spectra of ^{195}Pt for the species

$[\text{Pt}(\text{NH}_3)_2(\text{OH})(\text{H}_2\text{O})]^+$, $[\text{Pt}(\text{NH}_3)_2(\text{OH})]_2^{2+}$ and $[\text{Pt}(\text{NH}_3)_2(\text{OH})]_3^{3+}$. In the blood substitute Hanks medium [38], conversion of *cis*- $[\text{Pt}^{15}\text{NH}_3)_2(\text{H}_2\text{O})_2](\text{NO}_3)_2$ into *cis*- $[\text{PtCl}_2(^{15}\text{NH}_3)_2]$, *cis*- $[\text{PtCl}(\text{H}_2\text{O})(^{15}\text{NH}_3)_2]^+$, $[\text{Pt}^{15}\text{NH}_3)_2(\text{OH})]_3^{3+}$ and $[\text{Pt}^{15}\text{NH}_3)_2(\text{OH})]_2^{2+}$ have been observed, the reaction taking several hours at 30°C. In 0.15 M NaCl, both $[\text{Pt}(\text{NH}_3)_2(\text{OH})]_3^{3+}$ and $[\text{Pt}(\text{NH}_3)_2(\text{OH})]_2^{2+}$ are converted overnight into *cis*- $[\text{PtCl}(\text{H}_2\text{O})(\text{NH}_3)_2]^+$. Indications that the hydrolysis products of cisplatin may in fact be responsible for some of its toxic effects in vivo, notably nephrotoxicity [39], have stimulated further studies.

At physiological pH the predominant hydrolysed species is *cis*- $[\text{Pt}(\text{NH}_3)_2(\text{H}_2\text{O})(\text{OH})]^+$, which has been shown to oligomerize quite rapidly. These oligomeric species are toxic and might be responsible for the toxicity of cisplatin. Chelates in which NH_3 ligands have been replaced by 1,2-diaminocyclohexane (dach) seem to show higher antitumour activity than cisplatin with reduced toxicity. The hydroxo-bridged dimers and trimers of dach-Pt(II) complexes are active anticancer agents and are less toxic than the monomer [40] in contrast to the NH_3 oligomers. The antitumour activity of the complex $\text{Pt}(\text{en})\text{Cl}_2$ (en = ethylenediamine) is well known and it is also less toxic than cisplatin [41].

The crystal structure of the hydroxo-bridged platinum(II) tetramer, cyclotetrakis(μ -hydroxo)tetrakis(ethylenediamine)platinum(II) tetranitrate has been determined by X-ray diffraction [42]. Each platinum in the hydroxo-bridged tetramer has square-planar coordination. The four platinum atoms in the ring are planar with two cross-ring oxygen atoms on one side of the plane and the other two oxygen atoms on the other side. The cross-ring O—O interactions seem to indicate intramolecular hydrogen bonding. The crystal is stabilized by an extensive hydrogen-bonding system. All the amine groups are hydrogen bonded to the nitrate ion.

Structurally the dimers are planar complexes containing a strained four-

membered  ring. The strain present in the ring has a pronounced

effect on the ^{195}Pt NMR resonance of the complex, causing the signal to appear about 500 ppm to lower field relative to those of the strain-free monomer and trimer [37,43]. Although relatively little is known about the reactivity of the dimer, the complex reacts with DNA nucleobases, 1-methylthymine and 1-methyluracil, to yield novel compounds containing two *cis*- $\text{Pt}(\text{NH}_3)_2^{2+}$ units bridged via two base moieties [44,45]. It has also been reported that platinum(II) dimers containing NH_3 , EtNH_2 , and $^i\text{PrNH}_2$ can be oxidized with hydrogen peroxide to dinuclear platinum(IV) compounds [46].

Cisplatin and analogues react with nucleotides such as 5'-GMP, and as one would expect, the aquo species react much faster [47] with nucleic acids than the dichloro species. Preliminary studies [48,49] with cisplatin analogues have already shown that in the case of carboxylate ligands as leaving groups, reactions with nucleic acids in vitro proceed much more slowly. Even less is known about the kinetics of platinum(IV) drugs, although evidence is accumulating that binding to DNA occurs only after reduction of platinum(IV) to platinum(II) species [27,50]. Much more experimental work is needed in this area of the kinetics of the hydrolysis processes of platinum(IV) complexes. Owing to its excellent solvent properties, dimethyl sulphoxide, Me_2SO , is commonly used to dissolve compounds in biological studies. Kinetic studies reveal that Me_2SO substitutes for a single chloride ligand for both *cis*- and *trans*-DDP and further reaction of *cis*-DDP with Me_2SO results in the formation of several products in which Pt-NH₃ bonds are broken owing to the kinetic *trans* effect of sulphur-bound Me_2SO ligands [51].

Studies of *trans*-DDP binding to calf thymus DNA and to the model DNA substrate d(GpTpG) revealed significant differences in the rate of platinum binding and in the spectrum of products obtained when the complex was dissolved in aqueous buffer vs. Me_2SO for only 5 min prior to dilution with aqueous media [52,53]. These observations require that in the interpretation of the results of biological experiments employing Me_2SO as a solvent for platinum complexes, due consideration be given to the likelihood that coordination of Me_2SO to platinum in the reactive species fundamentally alters the nature of the products formed with DNA [52].

Rochon and Melanson [54] attempted the preparation of hydroxo-bridged oligomers from the reaction of $\text{Pt}(N,N'\text{-dimethylethylenediamine})\text{X}_2$ with silver sulphate and isolated a sulphate monomer. The molecular and crystal structures of the platinum(II) complex with aquo and sulphate ligands, aquo(*N,N'*-dimethylethylenediamine)(sulphato)platinum(II) hydrate have been determined. The square-planar environment of the platinum includes the bidentate ligand, one molecule of water and a monodentate sulphate ion through an oxygen atom. The coordinated aquo ligand is involved in two hydrogen bonds where it donates the protons. The lattice molecule of water plays a crucial role in stabilizing the crystal.

(ii) Reactions of platinum compounds with nucleobases, nucleotides and DNA

The reactivity of cisplatin in a biological milieu is critically dependent on chloride concentration. Hydrolysis reactions of the kind shown in Fig. 3 produce aquo complexes that are kinetically more reactive than the chloro-hydroxo complexes. When the drug is administered, therefore, the high

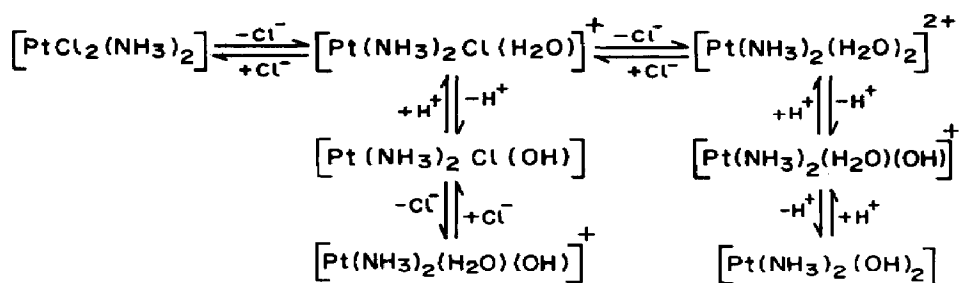


Fig. 3. Hydrolysis reactions of cisplatin.

chloride concentration (ca. 0.1 M) in blood suppresses its reactivity. After diffusion across the cytoplasmic membrane, the drug encounters a chloride concentration of 4 mM and reactions with biological targets can take place. It was postulated [55] that reactions of compounds such as *cis*-DDP with DNA bases and even DNA itself *in vivo* go through an aquation product which is supposedly *cis*-[Pt(NH₃)₂(H₂O)₂]²⁺ (Fig. 3).

The continued interest in the therapeutic potential of cisplatin and its analogues is reflected in the large number of new publications ranging from mechanisms of action to pharmacokinetic and clinical studies. Recent work confirms the likely importance of interactions with DNA or chromatin in inducing cytotoxic effects on cells, although it is still not evident which specific reaction(s), if any, is most significant. The need to monitor cisplatin concentrations *in vivo* has led to the development of many new sophisticated techniques for detecting platinum in body tissues or fluids and to many studies on the pharmacokinetics of cisplatin and its analogues. Pt-195m-labelled chloroammineplatinum(II) complexes have been synthesised [56] and used to study the distribution of these complexes in rats. The order of tissue retention at 24 h post-injection was kidney > liver > lung > genitals > spleen > bladder > adrenals > colon > heart > pancreas > small intestines > skin > stomach > brain.

Reaction products of cisplatin and deoxyribonucleosides, separated by high performance liquid chromatography (HPLC) and characterized by ¹H NMR, indicated monofunctional platination of N(3) of cytidine, N(7) of guanosine, N(1) of adenosine and N(7) of adenosine [57]. Bifunctional platination occurred with guanosine N(7)–N(7), adenosine N(7) to N(7) and adenosine N(1) to N(7). The mixed bifunctional products, N(7) of guanosine–N(1) of adenosine and N(7) of guanosine–N(7) of adenosine, were also obtained. Both 2:1 and 1:1 deoxyguanosine–cisplatin products and their acid hydrolysis products can be separated by cation exchange chromatography [58]. No evidence has been found for N(7)–O(6) chelation of guanine to platinum as previously proposed [59,60].

Early observations indicated that *cis*-[Pt(NH₃)₂Cl₂] preferentially attacks

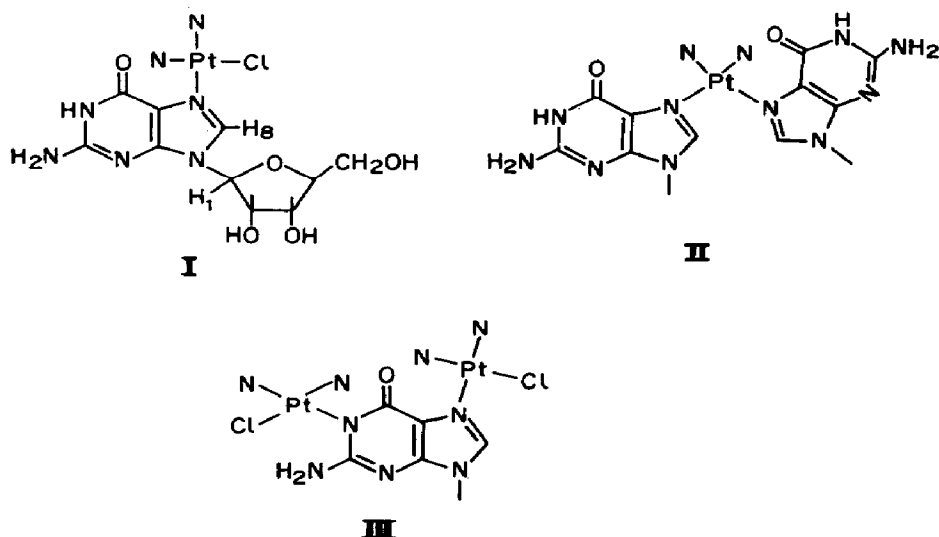


Fig. 4. Partial structures for species I, II and III. For I, the complete structure of guanosine is illustrated.

guanine residues in DNA [61–63]. Numerous investigations have more definitely confirmed, with a variety of experimental techniques, that guanine is attacked preferentially, and that in the major DNA adduct, both Cl ligands are eventually replaced either with guanine residues [64–67] or with one guanine and one adenine. However, Clore and Groneborn [68] reported observing three species I, II and III (Fig. 4) on treatment of 5'GMP with *cis*-DDP in the presence of 0.5 M KCl and excess platinum complex over 5'GMP (10:1). Species III, formed at long reaction times, was assigned a structure in which N(7) and O(6) were coordinated as a chelate to one platinum centre. Species I and II were both considered to be rotational isomers of cis -[Pt(NH₃)₂Cl(GMP)]⁻. ¹⁹⁵Pt and ¹H NMR spectroscopic characterization of the three species I, II and III formed between guanosine or 5'GMP and *cis*-DDP in a ratio of 1:10 with excess Cl by Miller and Marzilli [69] clearly ruled out the N(7)–O(6) chelate structure for III formed with 5'GMP (Fig. 5). However, a UV difference spectral study of the interactions between *cis*-dichlorobis(theophylline)platinum(II) with calf

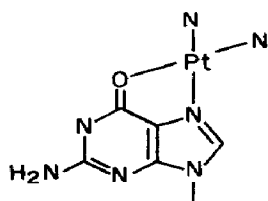


Fig. 5. Hypothetical structure for N(7)–O(6) chelate with N(1) deprotonated.

thymus DNA implicated binding of N(7), O(6) positions of the guanine residues of DNA to the metal [70].

Palladium complexes are often considered to be good structural models for platinum complexes and the existence of an N(7)–S(6) chelate in $\text{Pd}(6\text{-mercapto-9-benzylpurine})_2$ [71,72] is known. Also extended X-ray absorption fine structure (EXAFS) was presented to support the existence of such chelate formation (N(7)–S(6)) in the structures of $\text{Pt}(6\text{-mercaptapurine riboside})_2$ and $\text{Pt}(2\text{-amino-6-mercaptapurine riboside})_2$ [73].

Recent theoretical studies [74] have confirmed the strong thermodynamic preference for guanine-N(7). The kinetic preference might be related to the attractive hydrogen bonding at O(6). However, this binding is generally accepted to be highly improbable [75], though a hydrogen-bond-accepting role of the O(6) group is quite probable [76,77] and is in fact clear from several X-ray studies [78] of solid products.

Detailed studies of hydrolysis and ligand exchange kinetics [79–81] with 5'GMP and 5'AMP and diadenosine tetraphosphate have illustrated that kinetics might be much more important than previously thought. A ^1H NMR study of the reaction of 5'GMP with $\text{cis-}[\text{Pt}(\text{ND}_3)_2(\text{D}_2\text{O})(\text{OD})]^+$ at pD 6.8 shows the formation, as the predominant product, of N(7)-bound $\text{cis-}[\text{Pt}(\text{ND}_3)_2(\text{GMP})_2]^{2+}$. The NMR spectra recorded as a function of time at various concentration ratios show two kinetically allowed intermediates along the reaction course. The first is an N(7)-bound complex $\text{cis-}[\text{Pt}(\text{ND}_3)_2(\text{GMP})(\text{OD})]^+$ and the second can best be interpreted as $\text{cis-}[\{\text{Pt}(\text{ND}_3)_2\}_2(\text{GMP})_2]^{4+}$ in which each platinum atom is coordinated to N(7) of one 5'GMP molecule and to O(6) of the other. In parallel experiments using $\text{trans-}[\text{Pt}(\text{ND}_3)_2(\text{D}_2\text{O})(\text{OD})]^+$ on the 5'GMP, the second intermediate is not found.

The first crystallographic evidence for the formation of a balanced N(7), O(6) chelate with a 6-oxopurine was reported by Cozak et al. [82]. The compound $\text{bis}(\eta^5\text{-cyclopentadienyl})(\text{theophyllinato})\text{titanium(III)}$ was prepared using either $(\eta^5\text{-C}_5\text{H}_5)_2\text{Ti}(\text{CO})_2$ or $(\eta^5\text{-C}_5\text{H}_5)_2\text{TiCl}$ as a part of a research programme to study the interactions of the titanocene moiety $(\eta^5\text{-C}_5\text{H}_5)_2\text{Ti}$ with DNA. The dichlorometallocenes of titanium, vanadium and niobium and the ferrocenium cation belong to one of the most important classes of metal agents, after platinum complexes, showing promising antitumour properties. The titanocene structure is preserved in the complex. The $(\eta^5\text{-C}_5\text{H}_5)_2$ unit has the usual "open-clamshell" arrangement with titanium-centroid distances of 2.054 and 2.073 Å and an angle of 135° between the titanium-centroid direction. The two remaining sites of the approximate tetrahedron are occupied by the N(7) and O(6) atoms of deprotonated theophylline with Ti–N(7) and Ti–O(6) distances of 2.211(3) Å and 2.278(2) Å respectively. The EPR spectra of this compound are

consistent with the same structure being retained in toluene–benzene solutions. It appears that the overall environment of the coordination sites plays a determining role in the formation of the chelate ring by assembling two sterically complementary fragments. The fact that chelation has not been observed so far suggests that N(7),O(6) may be disfavoured under less restricted conditions. However, the possibility of such a ring being formed should not be dismissed, even for platinum, since Lippert and coworkers [128] recently showed that platinum forms a four-membered chelate ring via N(3),N(4) with deprotonated 1-methylcytosine which has not been considered a likely possibility.

Very recently, using a semiempirical all-valence method, modified and extended to transition series elements, electronic structure and intermolecular interactions of the model antitumour platinum(II) compounds with guanine and thioguanine have been calculated [83]. It is concluded that *cis* platinum(II) complexes with guanine form stable intrastrand N(7),N(7) cross-links, but chelation to the O(6) atom is also possible. The *trans* isomers of platinum(II) exclusively form interstrand cross-links but the *cis* platinum(II) complexes with thioguanine form almost entirely N(7)–S(6) five-membered chelates.

Hard chemical evidence for the existence of N(7)–O(6) chelate formation with guanine is lacking at the present time. One aspect that makes such a proposition particularly attractive is the involvement of the O(6) site (of guanine). In the field of carcinogenesis by alkylating agents, it is suggested that alkylation at the O(6) site is the most relevant in causing mutation in somatic cells. This is considered to be a necessary step in the transformation of the normal cell into a cancer cell. When this lesion is not repaired prior to DNA replication, this leads to a mispairing with thymine instead of the correct pairing with cytosine. Further replication leads to the replacement of the original GC pair by an AT pair, a base substitution mutation. If the cancer cell becomes so because of its inability to repair the O(6) guanine lesion caused by a carcinogen, then it may also be unable to repair the cisplatin-induced damage [84]. However, the normal cells have intact repair mechanisms and can repair the damage prior to DNA replication and thus survive. This postulate allows an explanation of the selective destruction of the cancer cells by the platinum drugs. *trans*-[Pt(NH₃)₂Cl₂] cannot form such a closed ring chelate involving N(7)–O(6) positions of guanine and this accounts for the difference between the biological effectiveness of the *cis* and *trans* isomers (*trans*-DDP is clinically ineffective).

¹H NMR studies of the reaction between *cis*-Pt(NH₃)₂Cl₂ (*cis*-Pt) and trinucleotides and tetranucleotides indicated the likely distortion induced in DNA by chelation of either adjacent guanines (d(GpG)–*cis*-Pt) or guanines separated by one base (d(GpCpG)–*cis*-Pt) [85,86]. Such a cross-link will

produce a very different lesion in DNA in comparison with cross-links via *cis*-Pt involving adjacent guanines. The latter interaction might well promote the formation of Z-DNA-like conformations at neighbouring sites along the DNA strand. Substantial conformational changes were reported [85,87] accompanying *cis*-Pt binding upon reaction with $[d(\text{ApGpGpCpT})]_2$. Platination of the guanosine monophosphates affects the sugar conformational equilibrium to favour the *N* conformation of the deoxyribose ring. This feature is also apparent in ribose mononucleotides and is possibly caused by anomeric effects. In *cis*- $[\text{Pt}(\text{NH}_3)_2\{d(\text{pG})\}_2]$ the phase angle of pseudorotation of the *S*-type sugar ring is 20° higher than that of the free $d(\text{pG})$, which might be an indication for an ionic interaction between the positive platinum and the negatively charged phosphate [85]. Cross-links between polydeoxyguanylic acid and polydeoxycytidylic acid occur with a frequency of one cross-link per 50–67 platinum reactions between N(1) of guanine and N(3) of cytidine. However, an attack of cisplatin on N(7) of guanine in DNA or poly(dG)–poly(dC) may be initially required in order to cause the required partial deprotonation of N(1) of guanine [88]. Local regions of denaturation are present in calf thymus DNA treated with cisplatin but not in DNA treated with *trans*-DDP [89].

The two main products (total yield greater than 95%) that are obtained in the reaction of *cis*-DDP with the trinucleotide $d(\text{GpApG})$ [90] have been characterized by high field ^1H NMR spectroscopy and by analysis of their enzymatically digested products with anion exchange chromatography (FPLC) and platinum atomic absorption spectroscopy. The results indicate the formation of both *cis*- $\text{Pt}(\text{NH}_3)_2[d(\text{GpApG})-\text{N}(7)1, \text{N}(7)3]$ (yield, 80%) and *cis*- $\text{Pt}(\text{NH}_3)_2[d(\text{GpApG})-\text{N}(7)2, \text{N}(3)3]$ (yield, 20%). No influence due to temperature or prior hydrolysis of *cis*-DDP was observed on the product ratio. The observation that only an AG chelate but no GA chelate is formed agrees with other studies in which only an AG chelate is reported.

Crystals [91] of the adduct of the anticancer drug *cis*-DDP with $d(\text{pGpG})$, its putative target on DNA in the cancer cell, have been obtained and used in an X-ray crystallographic study to elucidate its molecular structure. Each of the four crystallographically independent *cis*- $[\text{Pt}(\text{NH}_3)_2(d(\text{pGpG}))]$ molecules comprises a square-planar platinum atom bonded to two ammonia ligands and two N(7) atoms of guanosine nucleoside from the same chain. Base stacking of the two adjacent guanine rings is completely disrupted by coordination to the *cis*- $[\text{Pt}(\text{NH}_3)_2]^{2+}$ unit.

The nature of the adducts formed between DNA and *cis*-DDP and which of these accounts for the drug's cytotoxic properties have been the subject of several investigations. Many types of interactions have been proposed: DNA-interstrand cross-links, DNA-intrastrand cross-links and cross-links between DNA and proteins. Evidence for the existence and biological

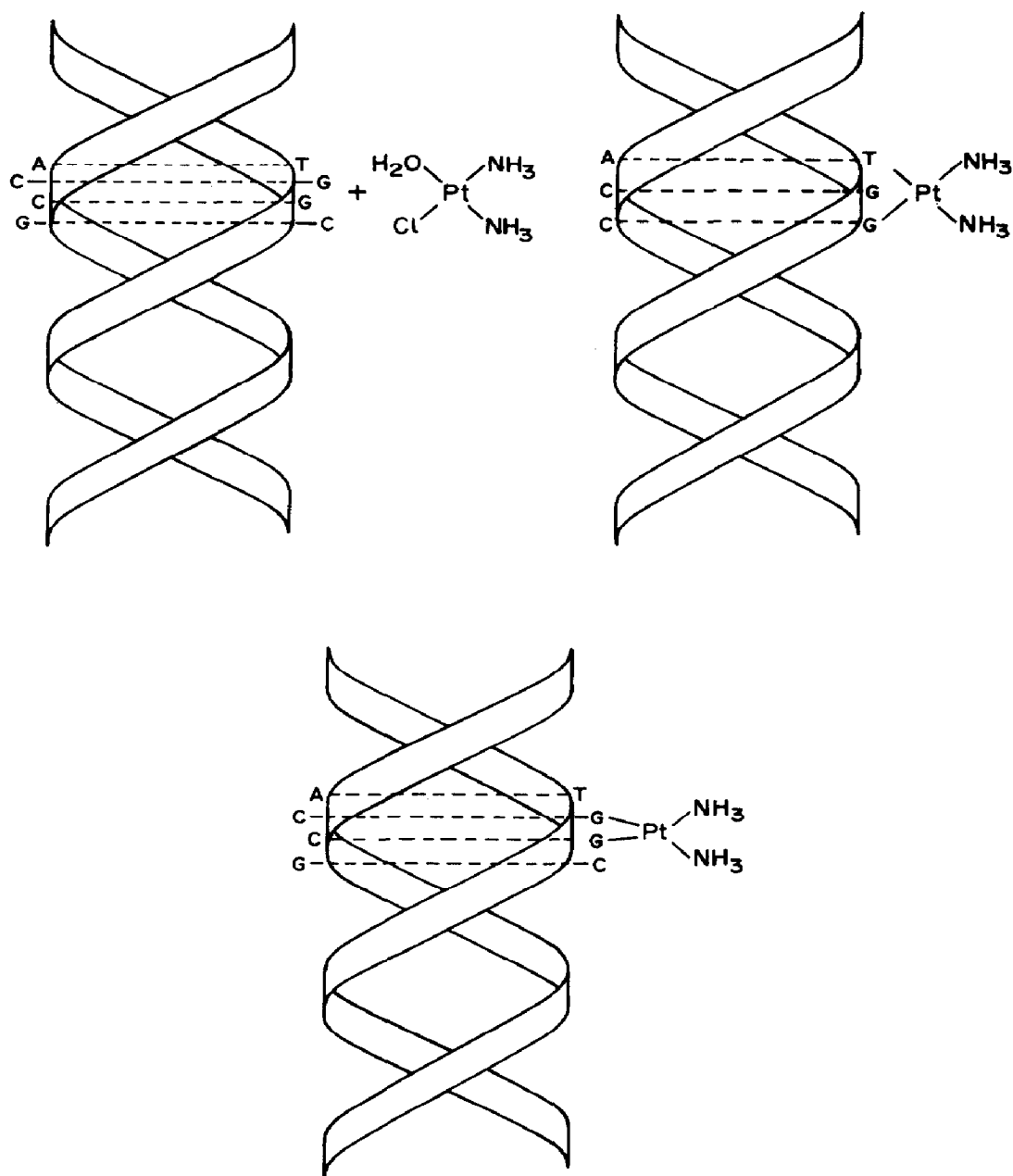


Fig. 6. Schematic binding possibilities for cisplatin to double-stranded DNA.

relevance of each of these classes of adducts have been discussed in detail by Pinto and Lippard [92] and Reedijk [93,94].

There are two possible interactions for platinum complexes with DNA. The first is a monofunctional interaction that does not affect the DNA structure. The second is a bifunctional interaction that appears to denature

the DNA double helix locally. Figure 6 shows a scheme by which cisplatin interacts and causes local changes in the DNA double-helical configuration. Cisplatin reacts with water to form a reactive aquated species that interacts with guanosine in native DNA to form a covalent linkage of cisplatin to DNA. As a result of this binding, hydrogen bonds between guanosine and cytidine on opposite DNA strands are broken. The cisplatin molecule then reacts with an adjacent nucleoside (bifunctional binding) to cause further hydrogen-bond breakage, resulting in local denaturation of the DNA double helix. These alterations in the DNA structure probably affect the fidelity of enzymatic DNA replication and RNA transcription leading to lethal events in the cell. Other studies have implicated cisplatin interactions with various enzymes including dehydrogenases in events leading to cytotoxicity.

Complexes [95] formed in vitro between *cis*- or *trans*-Pt(NH₃)₂Cl₂ and DNA were found to contain monofunctional adducts that reacted with exogenous guanine. ¹⁴C-labelled guanosine bound irreversibly to *cis*- and *trans*-DDP-DNA complexes to form bisguanine adducts. The reaction was first order with respect to concentration of both ¹⁴C-labelled guanosine and the Pt-DNA complex, but the rate of the reaction varied non-linearly as a function of the level of platinum binding on DNA. The concentration of the monofunctional adducts was highest immediately following reaction of *cis*-DDP with DNA for 2 h at 37°C, at which time they represented more than 15% of the *cis*-DDP-DNA lesions. The *cis*-DDP-DNA complex reacted with ¹⁴C-labelled guanosine by two kinetically distinct processes indicating two types of reactive adducts. The most reactive adduct represented 5% of the platinum lesions. These monofunctional adducts disappeared during incubation of the Pt-DNA complexes in the absence of the drug, probably as a result of chelation to DNA.

A radio-labelled analogue of the cancer chemotherapeutic drug *cis*-DDP, has been used by Eastman [96] to determine the sites of platination in DNA. The drug [³H] *cis*-dichloro(ethylenediamine)platinum(II) (*cis*-DEP) was also incubated with DNA, defined nucleic acid heteropolymers and dinucleoside monophosphates. At low levels of modification of DNA, more than 50% of the lesions were attributed to an intrastrand cross-link between two neighbouring guanines, enzymatic removal of the phosphate between two nucleosides being inhibited by the complex. At higher levels of modification, these sites became saturated, and pronounced reaction occurred at several other sites. One of these represented an intrastrand cross-link between neighbouring adenine and guanine. Reaction was also demonstrated between two guanines separated by a third base, the latter being removed during digestion. This was a relatively minor adduct. More frequent was an intrastrand cross-link between adenine and guanine separated by a third base. The third base was retained during digestion. These trinucleotides were

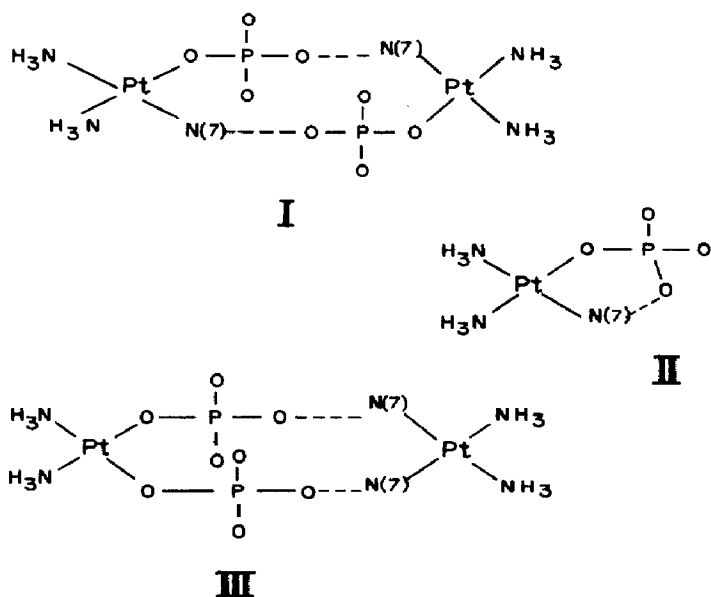
to contain either adenine, cytosine, guanine or thymine as their middle base. A specific orientation in the DNA was also observed with adenine always at the 5'-terminus. An additional more hydrophilic adduct was identified by denaturation studies as an interstrand cross-link but it represented a maximum of 1% of the total platination. A small proportion of monofunctional adducts, predominantly deoxyguanosine dependent were also detected. Both the drugs *cis*-DDP and *cis*-DEP reacted at identical sites.

DNA-interstrand cross-links (less than 1% of the total platination) have been characterized [97]. A cross-link between two deoxyguanosines was observed to be the most prominent adduct. It was proposed that the major sequence in which this cross-link occurs is 5'-CG-3'. Intrastrand cross-links represent the majority of modifications in DNA resulting from interaction with the cancer chemotherapeutic drug *cis*-DDP. These adducts were recently characterized, although several discrepancies remain to be resolved. In the re-evaluation studies [98] of interaction of *cis*-DDP with DNA, [^3H] *cis*-DEP was used because of the convenience of the radio label. Both drugs platinate the following sequences in DNA: GG 65%, AG 25%, GNG 6%. The frequency of platination at GG was too high to be explained by initial monofunctional platination at any guanine. The monofunctional adducts slowly rearranged to bifunctional adducts. It is suggested that this evolution of adducts may result from the drug "walking" along the double helix, a phenomenon that does not appear to occur in single-stranded DNA.

Model studies with dinucleotides [99–101] and with larger nucleotides [102–106] concluded that platinum chelation by two adjacent guanines is a kinetically favoured pathway, giving a single G-N(7)–G-N(7) *anti,anti* complex [91,107,108]. Considering the structural analogy between AG and GA sequences, the absence of d(GpA) chelate in the digests of platinated DNA [96,109] asks for comparative data for the first G-N(7) platination step and for the chelation step of the two sequences. Dinucleotide studies have already revealed a striking difference between platinum chelation by the GC and CG sequences. While CpG and d(pCpG) give only one C-N(3)–G-N(7) chelate (with G *anti* and C flipping between the *syn* and *anti* conformations) [110], GpC and d(pGpG) give the two couples of GN(7)–CN(3) isomers (with G *anti* and G *syn*) [111]. A comparative study of the reactions of ribo-ApG and GpA with *cis*-DDP and its diaquo derivative [*cis*-[Pt(NH₃)₂(H₂O)₂](NO₃)₂] [112] was reported. ApG gives a single A-N(7)–G-N(7) chelate (less than 95%), *cis*-[Pt(NH₃)₂(ApGN(7)1N(7)2)]⁺, with an *anti,anti* configuration of the bases. GpA gives four isomeric platinum adducts, one G-N(7)–A-N(1) chelate (32% of the mixture) and three G-N(7)–A-N(7) chelates (5%, 42% and 21%); *cis*-[Pt(NH₃)₂(GpA-N(7)1,N(1))]⁺ presents a relatively fast rotation of the adenine about its glycosidic and N(1)–Pt bonds for the conversion of the major into the minor

conformer at 18°C. The results are attributed for the first step to charge interaction and/or hydrogen bonding between the cationic aquated platinum species and the monoanionic phosphodiester group, favouring N(7) platination of the 3'-purine. For the chelation step there is hydrogen bonding within the intermediate complex $cis-[Pt(NH_3)_2(H_2O)(ApG-N(7))]^+$, between the phosphodiester bridge and an ammine or aquo ligand of platinum. The latter interaction prevents G rotation and favours base stacking leading to formation of the single A-N(7)-G-N(7) *anti,anti* chelate.

Over the past few years, high resolution NMR spectroscopic studies have been carried out to provide insight into the interactions of $cis-Pt(NH_3)_2Cl_2$ with DNA. Recent X-ray crystallographic [91,113] and molecular mechanics [114–116] studies have also contributed to our understanding of the structural chemistry of *cis*-DDP interactions with oligonucleotides. A comprehensive review of the material is given by Sherman and Lippard [117]. Novel direct 1H and ^{195}Pt NMR evidence for slowly interconverting “head-to-tail” rotamers in the reactions of *cis*-DDP and 5'AMP was presented by Reily and Marzilli [118]. The strong preference of platinum complexes for G residues, especially in polynucleotides such as DNA, is now very well established as an experimental observation. The preference for G is not completely understood [119]. The next most reactive target is A and then C. Unless the amine ligands are bulky, $cis-PtL_2(6\text{-oxopurine-N}(7))_2$ (where L_2 is two unidentate or one bidentate ligand and the designation N(7) indicates binding mode; charges are omitted since these depend on pH) exhibits rapid rotation about the Pt–N(7) bond on the NMR time scale [120]. Where L_2 is bulky, restricted rotation is observed [121]. Comparison of the properties prevalent in platinum anticancer compounds led Reedijk and his coworkers to speculate that facile rotation about the Pt–N(7) bond may be important in forming the crucial DNA lesion responsible for platinum anticancer activity [120]. A corollary of Reedijk's hypothesis is that restricted rotation in PtA and PtC adducts inhibits formation of the type of lethal DNA lesion formed by PtG adducts. Conclusive 1H and ^{195}Pt NMR evidence is presented that $PtL_2(5'AMP-N(7))_2$ complexes and the analogous 5'dAMP compounds exhibit detectable restricted rotation about the Pt–N(7) bond (compounds are known to exhibit such restricted rotation only when L_2 is bulky). The high selectivity of platinum compounds for the G residue may have a steric origin. The results point to the importance of the 6-NH₂ group's steric size and suggest that high selectivity for G could be a consequence of the smaller size of the 6-oxo group. Steric effects of exocyclic groups are probably greater for N(1)-bound A and for N(3)-bound C derivatives. Rotation about the Pt–N(7) bond for 5'AMP *cis* to Cl (or H₂O) and NH₂ is facile as in analogous 5'GMP compounds. However, in bis-N(7),N(7) nucleotide complexes, rotation about the Pt–N(7) bond is detecta-



Scheme 1

bly impeded for 5'AMP but not for 5'GMP. In these simple nucleotide complexes the lowering of the pK_a of the OPO_3H^- group on 5'AMP *cis* to an NH_2 group points to the importance of hydrogen bonding between the NH and OPO_3^{2-} groups.

Multinuclear NMR studies [122] and the kinetics of the formation of the $Pt(II)$ -adenine nucleotide complex in solution suggest the existence of two products in solution. Both the phosphate group and N(7) of the purine ring are coordinated to the platinum atoms. The structures proposed for some of the probable complexes in solution, consistent with the observed stoichiometry (1 : 1), are shown in Scheme 1.

In structure I, the two AMP ligands are bridged between the two $Pt(NH_3)_2^{2+}$ units in a "head-to-tail" fashion such that each platinum atom is coordinated to N(7) of one nucleotide and to the phosphate group of the other. The N(7) sites for the two bridging nucleotides in structure III are bonded to one platinum atom while the two phosphate groups are coordinated to the other forming a "head-to-head" dimer. A chelate-type structure coordinating through the phosphate and N(7) site of the same nucleotide molecule is shown in structure II. The X-ray crystal structure of a dimer $[Pt(en)(5'CMP)]_2 \cdot 2H_2O$ has been reported by Louie and Bau [123] in which N(3) and phosphate oxygen are coordinated to platinum in a head-to-tail fashion. Strong hydrogen bonding between phosphate oxygen and a hydrogen of the amine group was observed. Structures I and II are regarded to be the most likely structures for the observed products. Also, the UV difference spectral data of interactions, in solution, of the platinum(IV) and

palladium(II) complexes *cis*-dichloro-bis(theophyllinato)-*trans*-dihydroxy-platinum(IV) (*cis*-Pt(theoph)₂Cl₂(OH)₂) and *cis*-dichlorobis(cytidine)palladium(II) (*cis*-Pd(Cytd)₂Cl₂) with calf thymus, imply binding through N(7) of the guanine residue and the phosphate oxygen in a chelate form [124].

The reactions of 5'GMP with a series of (1,3-diamine) compounds containing sterically bulky substituents were studied [125]. A bulky amine substituent in the platinum compound may induce conformational change of the DNA after platinum modification and may lead to a decrease and/or disappearance in hydrogen-bonding ability between the coordinated amino group and nucleic acid constituents (bases, phosphates). Further, the presence of a substituent may lead to restricted rotation of 5'GMP about the Pt–N(7) bond and retardation of the reaction rate between 5'GMP and Pt(1,3-diamine). The reaction between 5'GMP and bifunctional platinum compounds occurs via a two-step mechanism. The first step corresponds to the formation of a 1:1 compound [Pt(diamine)(5'GMP-N(7))(OH₂)], and the second step to formation of a 2:1 compound [Pt(diamine)(5'GMP-N(7))₂]. The rate constants for each step were determined and discussed in relation to the nature of the substituent.

Using ¹H-detected heteronuclear multiple quantum coherence (HMQC) two-dimensional correlation spectroscopy and a ¹⁷O/¹⁸O labelling technique, Byrd et al. [126] unambiguously assigned the ³¹P signals in the solution spectrum (15°C) of the adduct of d(TGGT) and Pt(en)Cl₂, d(TGGT)Pten, at – 2.88, – 4.17 and – 4.21 ppm to GpG, TpG and GpT respectively. The d(TGGT)Pten ¹H NMR chemical shifts are characteristic of *cis* platinum adducts in which platinum is bound to N(7) of adjacent guanosine [108,127] bases. The study, a new approach for assigning ³¹P signals, extends the apparent universality of the (GpG)Pt conformation to lower temperature, to an additional sequence and to a change in the amine moiety. Since GpG–Pt adducts are the major products from the treatment of DNA with platinum anticancer agents, it was inferred that adducts similar to d(TGGT)Pt(en) are at least partly responsible for the – 3.0 ppm resonance observed in DNA nucleosomes.

Metal binding can occur in principle at all sites having a lone pair of electrons. However, in practice, metal binding occurs only to a relatively small number of sites, i.e. cytosine-N(3), guanosine-N(7), adenosine-N(1) and N(7). Lippert and coworkers [128], however, have reported the first N(3),N(4) chelate of cytosine. The crystal structure data show that in the platinum(IV) complex, *trans,trans*-[Pt(NH₃)₂(C₅H₆N₃O₂)₂](NO₃)₂ · 2H₂O, platinum coordination is through N(3) of the cytosine ring and through the deprotonated amino group N(4) leading to two four-membered chelate rings about platinum. The platinum coordination is completed by two mutually *trans*-NH₃ groups which are at right angles to the chelate rings. This

complex demonstrates that N(4) deprotonation and metal binding occur in a condensation reaction involving a hydroxo ligand and even in acidic media. It also exemplifies the enormous angular strain platinum(IV) can withstand in order to accomplish chelate formation.

Although binding of metal ions to the sugar ring oxygen atoms has rarely been observed, Pt-phosphate interactions do occur in certain cases [122,129,130]. The role of phosphate under physiological conditions seems to be only secondary for coordination; nevertheless, it is quite important for hydrogen bonding. One of the reaction products of aquated *cis*-PtA₂ (A = NH₃, NH₂CH₃) with 5'GMP, 5'dGMP, 5'IMP or 5'dIMP has spectral characteristics previously interpreted [81] as evidence for dimers such as *cis*-[Pt(NH₃)₂μ-(5'GMP-N(7),O(6))₂] (because of the dependence of the complex charge on pH the charges are omitted). Definitive NMR evidence was presented [130] that this species is in fact the N(7),PO chelate, (N(7),αPO₄), *cis*-[Pt(NH₃)₂(5'GMP-N(7),PO)] and that this is in equilibrium with *cis*-[Pt(NH₃)₂(GMP-N(7)(H₂O))] and is best illustrated by a study of the equilibrium between the species *cis*-[Pt(NH₂CH₃)₂(5'IMP-N(7),PO)] and its aquated counterpart where the CH₃ and H₂ ¹H NMR signals provide deeper insight into the nature of this unusual compound. There has been much speculation over the existence and biological role of macrochelate metal complexes of 5'ATP and other 5'-nucleotide triphosphates. Marzilli and coworkers [131] reported conclusive evidence from a multinuclear NMR study, supported by a molecular mechanics calculation, that in dilute (5–30 mM) neutral D₂O solutions, the preferred 1:1 complexes formed between *cis*-Pt(ND₂CH₃)₂(D₂O) and purine 5'-NTPs are monomeric macrochelates of the type *cis*-Pt(ND₂CH₃)₂(5'NTP-N(7),γPO) where the nucleotide is bound via N(7) and an oxygen atom of the γ-phosphate group. Such species were observed as intermediates during the course of the reaction when $r(\text{Pt}/\text{NTP}) = 0.5$ and were the major products formed at $2 \geq r \geq 1$. For 5'ATP, the addition reaction site at N(1) leads to ¹H NMR spectra indicative of polymerization when $r \geq 1$. A detailed analysis of the ribose coupling constants of the macrochelate indicates that it is flexible enough to maintain a normal $N \rightleftharpoons S$ equilibrium blend of sugar ring conformations with a slight shift towards the *N* conformer. Molecular mechanics calculations on the model, *cis*-Pt(NH₃)₂(5'GTP-N(7),γPO), demonstrated that these results are consistent with γ-phosphate coordination. A similar examination of the macrochelate formed with 5'dIMP, where only the α-phosphate group is available for coordination, revealed even more pronounced spectral effects indicating that the sugar ring clearly adopts an unusual conformation. Indeed, a second unambiguous N(7),αPO chelate Mo(C₅H₅)₂(5'AMP-N(7),αPO) was recently reported by Marks and coworkers [132]. The neutral complex Mo(C₅H₅)₂(5'AMP-N(7),αPO) is

stable enough to be isolated and characterized as a monomer by cryoscopic and mass spectral methods. It is also interesting to point out that $\text{Mo}(\text{C}_5\text{H}_5)_2\text{Cl}_2$ is an antineoplastic agent. The crystal structure of $[\text{Cp}_2\text{Mo}(5'\text{dGMP})]_2$ provides a further example of binuclear N(7),PO chelation [133].

Recent evidence suggests that the influence of the platinum adduct on the DNA structure may be an essential feature influencing the activity of the drug [92]. Since ^{31}P NMR spectroscopy is a structurally informative method that can be applied to both nucleosomes and oligonucleotides and since the ^{31}P signal position appears to be dependent on the state of DNA, Marzilli and coworkers [134] initiated studies aimed at evaluating the relationship between ^{31}P NMR shifts and the nature of the DNA. Initial ^{31}P NMR spectral studies of DNA treated with antitumour active platinum drugs revealed the presence of a new signal ca. 1 ppm downfield from the main DNA signal [135,136]. The downfield signal is not observed for DNA treated with antitumour-inactive platinum compounds such as *trans*- $\text{Pt}(\text{NH}_3)_2\text{Cl}_2$ [137]. A series of self-complementary oligomers varying in length from 8 to 14 bases with different sequences were treated with platinum drugs. Only when GpG was present in the sequence were downfield ^{31}P signals observed [138]. Separate studies of other small GpG-containing oligomers [106,139–141], both single stranded and self-complementary, indicate the presence of a platinum-intrastrand cross-link. In the earlier as well as in the present study of Pt–amine adducts of tetradexyribonucleotides (d(TGGT), d(GGTT) and d(pGGTT) and *cis*- PtA_2Cl_2 where A = en, $(\text{NH}_3)_2$, (MeNH_2) etc.), the downfield-shifted ^{31}P NMR signal appears to be characteristic of the intrastrand cross-link and no such signal was observed when the reactants, d(TGGT) plus *trans*- $\text{Pt}(\text{NH}_3)_2\text{Cl}_2$, could not form such an adjacent GG cross-link. The shift of the downfield ^{31}P NMR signal of the GpG moiety can be correlated with the potential hydrogen-bonding ability of the platinum moiety and of the oligonucleotide. In particular, if there is a phosphate group 5' to the GpG unit, the ^{31}P NMR signal is further downfield than in analogous species lacking such a group. Furthermore, when the amine group coordinated *cis* to the 5'G is capable of hydrogen bonding (e.g. NH_2), the GpG ^{31}P signal is further downfield than when this group is *cis* to amines incapable of hydrogen-bonding, e.g. Me_2N . The shift data indicate that the 5'-phosphate groups participate in hydrogen bonding. It may be argued that single-stranded oligonucleotides are not relevant to the anticancer activity of the platinum drugs and the study represents one of the initial steps in the analysis of the much more complex spectra typically found for duplexes.

The reactions of platinum compounds with d(pGpGpG) are interesting since in principle the formation of GG and GNG chelates is possible with

this trinucleotide. The main product of the reaction between cisplatin and the trinucleotide appeared to be [142] *cis*-Pt(NH₃)₂[d(pGpGpG)-N(7)1,N(7)2]. A minor product was assigned to be *cis*-Pt(NH₃)₂[d(pGpGpG)-N(7)2,N(7)3]. The characterization of the main adduct was possible by pH-dependent chemical shift data in combination with a comparison of the ribose H(1') splitting patterns. ³¹P PMR data were in agreement with a *cis* platinum chelate of neighbouring guanines. However, no indications of the so-called GNG adduct with this trinucleotide, i.e. *cis*-Pt(NH₃)₂[d(pGpGpG)-N(7)1,N(7)3], have been found.

The NMR spectral studies [143] of the major adduct, *trans*-[Pt(NH₃)₂{d(GpCpG)}], in the reaction of *trans*-DDP with the sodium salt of the deoxy trinucleoside diphosphate, d(GpCpG), reveal the structure to be comprised of an intrastrand cross-link between the N(7) atoms of guanosine nucleosides G1 and G3. The intervening cytidine nucleoside, in this adduct, is destacked and the G1 deoxyribose sugar ring switches its puckering from an *S*(C2'-*endo*) to an *N*(C3'-*endo*) conformation. This change in sugar pucker is similar to that observed for the 5'-nucleotide in *cis*-[Pt(NH₃)₂{d(GpG)}] [144] intrand adducts on DNA. This structural information is probably relevant to the therapeutic inactivity of *trans*-DDP.

The rate constants for the first platination of XpG and GpX ribodinucleotides (X = A, G, C) by *cis*-[Pt(NH₃)₂(H₂O)₂](NO₃)₂ (I) and [Pt(NH₃)₃(H₂O)](NO₃)₂ (II) complexes have been determined [144a]. The guanine platination by I is 2–9 times faster than by II and is accelerated by the presence of an adjacent G instead of A or C. These data were interpreted by modelling the pentacoordinated reaction intermediates using molecular mechanics. Ligand–nucleotide interaction seems to govern the transition state of the first platination step. In particular, with complex I, the influence of the adjacent guanine is due to hydrogen bonding between its O(6) and the non-leaving aquo ligand.

Platinum coordination compounds involving linked purines such as [1,3-bis(adenin-9-yl)propane] were investigated [145] as models for the interaction of platinum antitumour drugs with DNA and the molecular structure of the dimer bis[μ-1,4-bis(hypoxanthin-9-yl)butane]-bis-[(diaminoethane)platinum(II)]tetrakis(hexachlorophosphate)hydrate is reported. Each base in the ligand is coordinated to a different platinum atom via N(7). Each platinum atom coordinates to both nitrogen atoms of the diaminoethane ligand. The two units of dimer are related by an inversion centre, but each unit lacks the common C₂ symmetry often found with bis(purine) complexes. There is intramolecular hydrogen bonding between one O(6) and an adjacent nitrogen atom with an O–N distance of 2.89(1) Å. The pyrimidine moieties are on opposite sides of the coordination plane in the “head-to-tail” (htt) arrangement. This compound is thus the first *cis*-bis-N(7)-bound

6-oxopurine complex where only one O(6) participates in an intramolecular hydrogen bond. The results of this study greatly expand the range of conformational features found in models of the guanine–Pt–guanine cross-link. Several different intermediate conformations are likely to arise as the Pt–DNA interaction proceeds from the double helix to the final distorted conformation. The nature of the conformation in platinum oligonucleotide complexes needs to be further defined.

The differences in the binding of (1,2-cyclohexanediamine)platinum(II) isomers with d(GpG) were studied [146]. The ligand 1,2-cyclohexanediamine, *dach*, has three isomeric forms (*R,R*-, *S,S*- and *R,S*-*dach*) and the platinum(II) complexes of *R,R*-*dach* have a slightly higher antitumour activity than the corresponding complexes of *S,S*-*dach*. *R,S*-*dach* complexes are less active and less toxic. Optically active platinum(II) complexes are expected to interact with DNA in a different way since DNA has a chiral structure. The cyclohexane ring of $\text{Pt}(\text{R,S-dach})^{2+}$ is almost perpendicularly oriented with respect to the platinum-coordination plane. Conformations such as the torsion angle about glycosyl bonds and the puckering of furanose rings may be affected by the axially standing cyclohexane ring. Stereoselectivity observed in the experiments with DNA presumably arises from a steric hindrance caused by the axially standing cyclohexane ring.

The binding of the monofunctional compound $[\text{Pt}(\text{dien})\text{Cl}]\text{Cl}$ to the dinucleotides d(HpG) (H = A, C or T) has been studied separately and in competition experiments [147]. When using excess of (HpG) only, platinum coordination to the N(7) of the 3' guanine is observed as deduced from the chemical shift of the non-exchangeable nucleobase protons and their pH dependence. A kinetic effect of the 5'-nucleotide on the binding to the guanine appears to be present. The relative reaction rate of the platinum compound with dinucleotides decreases in the series, d(CpG), d(TpG) and d(ApG) (binding ratio, 4:3:2). Thus kinetic effects are indeed possible upon binding of platinum compounds to DNA as a result of neighbouring sequences. It is known that a 3'-terminal nucleotide in an oligonucleotide behaves differently from the other nucleotides. Moreover, the intramolecular stacking in the dinucleotides will be much smaller than when they are incorporated into larger double-stranded DNA fragments. *cis*-DDP has a varying reactivity and binding rate depending upon the choice of the nucleotide and upon the experimental conditions used, notably pH and the presence or absence of NaCl. Complexes formed in aqueous solution, between cisplatin or hydrolysis species and 5'AMP or 5'ATP, the latter with or without chloride ions, have been determined [148] using ^{195}Pt , ^{31}P , ^{13}C and ^1H NMR. The results lead to the conclusion that the only monodentate complexes with 5'AMP are *cis*- $\text{Pt}(\text{NH}_3)_2(5'\text{AMP-N}(7))\text{Cl}$ at acid pH and *cis*- $\text{Pt}(\text{NH}_3)_2(5'\text{AMP})(\text{OH})$ at neutral and basic pHs. Other bidentate

complexes identified were *cis*-Pt(NH₃)₂(5'AMP-N(7))₂ and *cis*-Pt(NH₃)₂-(5'AMP-N(7))(AMP-PO). In neutral and basic pH ranges, the phosphate moiety of 5'ATP is the most reactive site. Furthermore, under the experimental conditions used, neither the 5'ATP nor the 5'AMP have shown binding to N(1).

The conformational changes associated with the sugar portion of 5'GMPNa₂ and 3'GMPNa₂ have been reported [149] following interaction with several platinum complexes as evidenced by Fourier transform and ¹H NMR spectroscopy. In aqueous solution the conformational population of the 3'-*endo* form of the sugar moiety in bis-5'GMP-Pt(II) complexes and 3'GMPNa₂ is 40–50%, whereas in 5'GMPNa₂ it is 36% and in the *cis*-[Pt(NH₃)₂(3'GMP)₂]²⁺ complex it is 66%. It is suggested that platination or protonation at guanine-N(7) causes a change in the ribose ring conformation from C2'-*endo* to C3'-*endo* in 5'GMPNa₂, whereas in 3'GMPNa₂ and its complex *cis*-[Pt(NH₃)₂(3'GMP)₂]²⁺ such a sugar conformational change is much less prevalent. This is most probably due to the phosphodiester linkage at the 3'OH position of the sugar, which is not easily perturbed by platination at the N(7) site of guanine. The sugar moiety seems to be much less flexible when the phosphate group is at the 3'OH site of the GMP molecule. The ribose ring, however, of *cis*-[Pt(NH₃)₂(5'GMP)₂]²⁺ in the solid state adopts a predominantly C3'-*endo,anti* conformation. The conformational changes of the sugar ring in Pt-guanosine complexes have also been reported [150]. It appears that there is a tendency to increase slightly the C3'-*endo,anti,gg* sugar pucker in aqueous solution upon platination, whereas in the solid state such a tendency is much stronger. A study [151] concerning changes in the conformation of sugar in the dinucleotides suggested that the binding of the anticancer drugs (intercalating or chemically bound) with d(GpG), d(GpC) or d(CpG) sequences in DNA may destroy the backbone sugar conformation of DNA by changing the sugar pucker to accommodate the strain caused by the presence of the drug. The conformational changes in sugar pucker from C2'-*endo* to C3'-*endo* or vice versa take place to accommodate and stabilize the drug-nucleotide adduct.

The structure of the *cis*-diammine platinum adduct with DNA depends on the hydrogen binding of the polarized ammine ligand to nucleophilic sites in DNA. The possibility of water bridges increases the number of types of structure. Molecular mechanics modelling with kinked oligomer duplex models suggested [152] that both "direct" and "through-water" binding conformations to phosphate are possible. The intrinsic energetics of this binding have been modelled for the Pt(NH₃)₄²⁺ ··· H₂O ··· H₂PO₄⁻ cluster with valence SCF MO calculations. The binding of water to Pt(NH₃)₄²⁺ and H₂PO₄⁻ has also been reported for a variety of conformations.

The kinetics of changes in the secondary structure of DNA induced by

the binding of platinum compounds were investigated by circular dichroism (CD) spectroscopy [153]. Changes in DNA CD spectra upon binding with the bifunctional compounds *cis*- and *trans*-DDP had a biphasic character. The initial fast binding was in both cases manifested by a decrease in the CD band of DNA, reflecting the denaturing nature of the monofunctional attachment. The following slower CD spectral changes, which reflect a rearrangement of the monofunctional binding to bifunctional attachment, were different in the reactions of *trans*-DDP and *cis*-DDP. In the former case the denaturing effect continued to prevail, and the bifunctional binding of *cis*-DDP led to an increase in the DNA CD band, which indicated that lesions induced in the DNA molecule by the monofunctional binding were reversed and only small distortions of a non-denaturing character remained. Kinetic curves obtained for *cis*-DDP and *trans*-DDP on the basis of the polarographic determination of platinum bound to DNA, which does not differentiate between the monofunctional and bifunctional binding, correspond most probably to two parallel reactions at different types of DNA binding site.

Conformational alterations induced in DNA by the binding of various bivalent and tetravalent complexes were characterized by means of differential pulse polarography and CD spectroscopy [154]. At low binding levels the platination of DNA markedly influenced its polarographic behaviour. The binding of the active antitumour platinum complexes resulted in minor conformational changes in DNA when the double-stranded structure remained unaltered. However, the attack by inactive antitumour compounds induced more severe alterations which had the character of denaturation of longer regions of the DNA molecule. The antitumour tetravalent platinum complexes could react with DNA without their prior reduction to the bivalent state and may induce, in DNA, conformational changes similar to those produced by bivalent cisplatin.

In another study of conformational changes of DNA fragments from chicken erythrocytes modified by platinum compounds, *cis*-DDP, *trans*-DDP and chlorodiethylenetriaminoplatinum(II) chloride, Balcarova and Brabec [155] observed by CD spectroscopy that the binding of the platinum complexes to *B*-DNA lowers the conformational freedom of DNA, so that it cannot acquire the *A*-conformation.

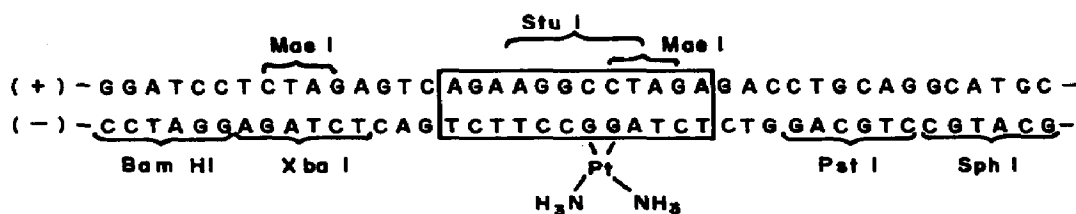
The self-complementary deoxyribohexanucleosidepentaphosphate, d(ApGpGpCpCpT) and the major product of its reaction with the antitumour drug, *cis*-DDP, have been characterized by high resolution ^1H and ^{31}P NMR spectroscopy [156]. Temperature dependence studies of the amino, imino and non-exchangeable base protons of $[\text{d}(\text{ApGpGpCpCpT})]_2$ indicate that the oligonucleotide adopts a duplex structure in solution with frayed terminal A1 · T6 base pairs. The results demonstrate that the isolated product

contains an intrastrand cross-link between two N(7) atoms of adjacent guanosine residues, the most prevalent product formed by *cis*-DDP in tumour cells. No evidence was found for the other commonly occurring adduct, an intrastrand d(ApG) cross-link. The data further indicate that *cis*-[Pt(NH₃)₂-{d(ApGpGpCpCpT)-N(7)-G2-N(7)-G3}] adopts a single-strand base-destacked coil structure that is unable to form a Watson-Crick duplex with itself. The results of this investigation agree with in vitro enzymatic and immunological studies implicating an intrastrand nearest-neighbour *cis*-Pt(NH₃)₂-guanine-N(7),guanine-N(7) cross-link as the most important structure in the biochemistry of cisplatin with DNA. Immunochemical studies have demonstrated the existence of this adduct and the related d(ApG) cross-link in the ascites fluid of L1210 tumour-bearing mice treated with *cis*-Pt(NH₃)₂Cl₂ and in the white blood cells of cancer patients treated with *cis*-DDP [157,158]. Furthermore, since biologically inactive *trans*-Pt(NH₃)₂Cl₂ is unable to form such adducts, this lesion uniquely demonstrates the requirement of *cis* stereochemistry. A deeper understanding of the molecular mechanism of the action of *cis*-DDP will require detailed investigation of the dynamic structural changes induced by both *cis*- and *trans*-Pt(NH₃)₂Cl₂ upon binding to duplex oligonucleotides and the ability of the cellular repair process to recognize and eliminate these adducts.

Mathematical models have been constructed [158(a)] to describe the kinetics of formation of *cis*-[Pt(NH₃)₂{d(...pApGp...)}] and *cis*-[Pt(NH₃)₂{d(...pGpGp...)}] from DNA and cisplatin-type systems; the rate constant and the entropy of activation of the initial reaction with G indicate that the 5'-phosphate is very important kinetically.

Wenxia et al. [158(b)] studied the interaction of cisplatin with the constituents of DNA by ¹³C NMR and CNDO/2 methods and the study indicates a new intrastrand cross-linkage formed between cisplatin and DNA through the N(7),N(1) atoms of two adjacent guanines. The results imply that the binding of platinum to the N(7) atom of neighbouring guanines on the same strand of DNA only weakens the hydrogen bonds between the GC base pair but does not rupture them. However, when platinum is bonded to neighbouring guanines on the same strand of DNA through N(7),N(1), the formation of hydrogen bonds and consequently of GC base pairs is all prevented. The formation of intrastrand cross-linkage of two adjacent guanines through N(7),N(1) by cisplatin, however, has not been found in a ¹H NMR study of oligonucleotide-cisplatin adducts and in enzymatic digestion studies. A possible reason may be that the mole ratio of cisplatin to oligonucleotide in the reaction system is too low, and the rate of N(7),N(1) cross-linkage is comparatively lower than that of N(7),N(7) cross-linkage.

The heterogeneity of reaction products of the globally platinated DNAs

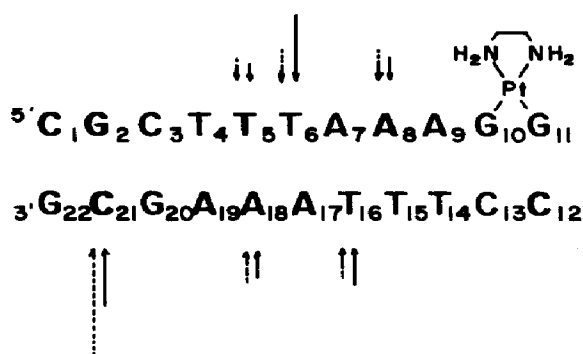


Scheme 2

has made it difficult to discern the effects of any one specific adduct upon the processing of DNA *in vivo*. Lippard and coworkers [159] recently achieved the synthesis and preliminary characterization of duplex bacteriophage an intact viral genome, M13 DNA containing a *cis*-[Pt(NH₃)₂d(GpG)] cross-link at a unique programmable site in the genome.

The material is prepared by ligating a chemically synthesized oligonucleotide containing the desired platinum adduct into a gapped duplex obtained by recombinant DNA technique. The resulting site-specifically-modified genome can be then used for various repair, mutagenesis and survival studies both *in vitro* and following its introduction into cells by transformation, *in vivo*. The first adduct, built into DNA from bacteriophage M13, is shown in the box in Scheme 2. The presence of platinum inhibits cleavage of the modified DNA by restriction endonucleases, *Stu* I and *Mae* I (in the box), but not by *Bam* HI, *Xba* I, *Pst*I, *SpH* I or *Mae* I (outside the box). Thus structural distortions induced in DNA by the *cis*-[Pt(NH₃)₂-d(dGpG)] intrastrand cross-link are fairly localized to within one helical turn from the site of platination. Addition of cyanide to the modified M13 DNA removes platinum as Pt(CN)₄²⁻ and restores sensitivity to cleavage by restriction enzymes. The construction of other such site-specifically-modified genomes, with different sequences and platinum complexes, and an examination of their biological properties *in vivo* should enable firm conclusions to be drawn about the relevance of different adducts to the mechanism of action of platinum anticancer drugs.

Bleomycin is commonly used therapeutically in combination with cisplatin. It has been observed that platination of DNA caused major alterations in the sequence specificity of Fe · BLM A₂ mediated cleavage, including the appearance of new cleavage sites and the suppression of others. To understand better the molecular basis of altered DNA cleavage, Gold et al. [160] studied a well-defined system d(CGCT₃A₃G₂) and oligomers d(C₂T₃A₃GCG) with a strong BLM cleavage site and a separate preferred platination site. It was anticipated that Fe · BLM-mediated modification of this duplex would occur primarily at C21 [161,162] and platination preferably at G10G11. In fact, treatment with one equivalent of hydrated *cis*-dichloro(ethylenediammine)platinum(II) gave quantitative platination of the



Scheme 3. Fe·BLM-mediated strand scission of a platinated ($\uparrow$) and non-platinated ($\uparrow$) DNA oligomer. The extent of cleavage at individual positions is proportional to the length of the arrows.

oligomer, more than 90% of which was localized at G10G11. As expected on the basis of work with 5'd(CGCT₃A₃CGC), treatment of the unplatinated undecanucleotide with Fe·BLM A₂ resulted in modification at C21 (Scheme 3). Analogous cleavage of the platinated oligomer afforded essentially the same total amount of products, but the ratio of products formed from C and T differed dramatically (2.2 vs. 0.7). Gel sequence analysis [163] of the platinated oligomer revealed a threefold increase in BLM-mediated damage at T6, which became the primary site of damage; a significant increase in cleavage at T16 was also noted. The emergence of TT as the preferred Fe(II)·BLM cleavage site is unprecedented and provides an opportunity to identify those features in DNA which render the duplex susceptible to Fe·BLM-mediated modification. This study extends the earlier observations in important ways, including better definition of the spatial relationships between the platinum centre and the BLM cleavage sites that lead to altered cleavage patterns, and shows that conformational alteration in DNA can lead to novel BLM-mediated DNA cleavage patterns and that platination does not alter the chemistry of DNA cleavage.

Direct crystallographic evidence for the intrastrand cross-linking in nucleic acid (tRNA) by *cis*-[Pt(NH₃)₂Cl₂] at four binding sites has been reported [164]. The interpretation of the result is that the *cis*-[Pt(NH₃)₂]²⁺ moiety forms an intrastrand cross-link at certain GG and AG sites within tRNA thereby causing a local distortion of the macromolecule. Features common to each of these binding sites are that at least one G residue is involved, that the adjacent bases in the native undistorted macromolecule are stacked on top of one another and that the N(7) donor atoms of these bases are ca. 4 Å apart and are not themselves involved in hydrogen-bonding interaction elsewhere in the macromolecule. Binding at one CG site is observed and a base flip by the C residue is invoked to rationalize the drug binding at this

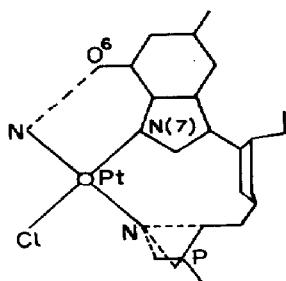
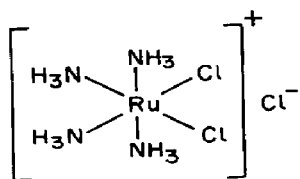
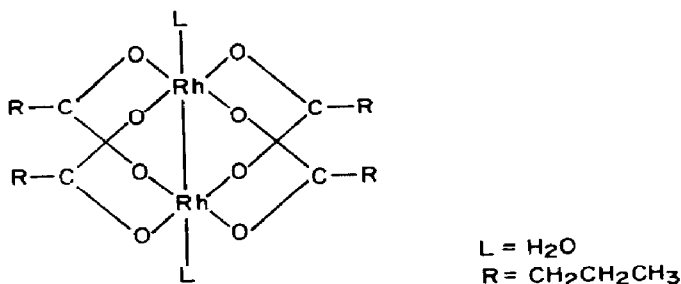


Fig. 7. The binding sites of *trans*-Pt(NH₃)₂Cl₂ to the guanosine-34 residue of phenylalanine tRNA.

cis-Tetrammine dichlororuthenium(III) chloride



Diaquotetrakis(μ -butyrato)rhodium(II)



1,2-Diaminocyclohexane(malonato)palladium(II)

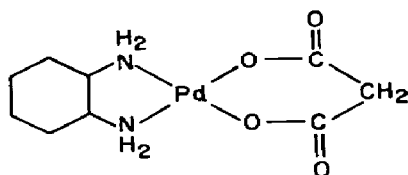


Fig. 8. Structures of ruthenium, rhodium and palladium complexes having antitumour activity.

site. A preference for the drug to bind at AG rather than at GA sites is noted.

It is worth noting that *trans*-[Pt(NH₃)₂Cl₂] was used as a heavy-atom label in the solution of the structure by X-ray diffraction of the phenylalanine-tRNA [165]. The results show that the *trans* isomer lost one chloride

ion and became bound to the N(7) position of G34 (Fig. 7). Interestingly, one of the ammine groups makes a hydrogen bond to the O(6) position of the same base whereas the ammine group *trans* to it can make three hydrogen bonds to the phosphate groups.

A recent study [166] of the kinetics and mechanism of the fundamental substitution processes during complex formation of the (diethylenetriamine)palladium(II) centre with nucleic bases, nucleosides and 5'-nucleotides in aqueous solution, reveals that contrary to normal square-planar substitution, the solvolysis of $\text{Pd}(\text{dien})\text{Cl}^+$ is not the rate-determining step but instead it is the subsequent anation of $\text{Pd}(\text{dien})\text{OH}_2^+$. The anation mentioned, reverse aquation and other subsequent reactions are expected to play an important role in the intimate mechanism of the interaction of related antitumour complexes with nucleic acid moieties. The approach, for instance, to slow down the reaction of *cis*- $[\text{Pt}(\text{NH}_3)_2\text{Cl}_2]$ with AMP and GMP by studying them in a large excess of chloride ion had been based on the incorrect supposition that hydrolysis of *cis*-DDP is the rate-determining step and would be hampered by high chloride ion concentration.

Scovell et al. [167] recently reported an interesting cross-linking reaction of cisplatin in the micrococcal nuclease accessible region of chromatin. The reaction of *cis*- $[\text{Pt}(\text{NH}_3)_2\text{Cl}_2]$ with chicken erythrocyte nuclei produces covalent cross-linking of high mobility group (HMG) proteins 1,2 and E to DNA, in addition to cross-links observed in low mobility group proteins. The preponderance of evidence presented in their communication suggests that the major site of *cis*-DDP binding in DNA is N(7) of the guanine residue in the major groove of DNA and the *cis*-DDP forms a second bond to the DNA binding domain of HMG 1 and 2 in the internucleosomal regions of chromatin. The *cis*-DDP binds to an amino acid residue within or in the vicinity of an α -helical segment of the protein. However, the nature of the local disruption of the DNA structure induced by HMG binding itself is yet to be defined.

C. MECHANISM OF ACTION OF SOME SECOND GENERATION PLATINUM DRUGS

It has been assumed that second generation platinum drugs which contain carboxylate ligands act by a similar mechanism to the chloride-containing drug cisplatin. Roos and Stokes [168] have investigated this by studying both the solution chemistry and the biology of two of the second generation drugs, carboplatin and ethylenediamine(malonato)platinum(II) (JM-40) and their chloro analogues cisplatin and ethylenediaminedichloroplatinum(II). The study includes the stability of the drugs, their reaction with chloride, methionine, glutathione and their reactions with DNA. In buffers that do

not react with these platinum drugs, there is no evidence of hydrolysis of the carboxylate complexes over a period of 72 h. The DNA damage produced by the cells is different for each drug, with cisplatin producing greatest damage. These results suggest that the carboxylate complexes may be metabolized differently from the chloro complexes and also that hydrolysis of the drugs is not an important step in their mechanism of action.

A comparative antitumour activity study [168a] on platinum(II) and platinum(IV) complexes containing 1,2-diaminocyclohexane (dach) ligand revealed that *trans,cis*-Pt(IV) (*SS*-dach)(OH)₂Cl₂ is more active than its mirror image, *trans,cis*-Pt(IV) (*RR*-dach)(OH)₂Cl₂, against L1210 leukemia implanted in mice. However, the activity is dependent on the tumour model, and against B16 melanoma implanted in mice, the activities of the two enantiomers are reversed with *trans,cis*-Pt(IV) (*RR*-dach)(OH)₂Cl₂ being more active than *trans,cis*-Pt(IV) (*SS*-dach)(OH)₂Cl₂.

D. STUDIES OF OTHER PLATINUM GROUP METAL COMPLEXES

Complexes of Group VIIIB metals, especially rhodium, iridium and platinum are reported [169] to have considerable antibacterial power. Palladium and platinum complexes of 6-mercaptopurine destroy some adenocarcinomas, and the complexes of dialkyl dithiophosphates reduce some mice tumours [170,171]. Platinum group metal complexes (of metals other than platinum) having antitumour activity are (1) *cis*-tetraamminedichlororuthenium(III) chloride, (2) diaquatetrakis(μ -butyrato)dirhodium(II) and (3) 1,2-diaminocyclohexane(malonato)palladium(II) [172]. They are illustrated in Fig. 8.

Work with nucleosides and nucleic acids indicates [173] that the coordination of ruthenium(II) to such ligands is similar to that of cisplatin or its analogues, so that analogous effects might be exerted on nucleic acid metabolism. Ammine ruthenium(III) ions, however, form unusual cytosinato and adenosinato species. Studies demonstrate that a number of ruthenium compounds serve as bacterial mutagens and so indicate that at least some ruthenium complexes are capable of damaging genetic material. The *in vivo* production of the more easily substituted ruthenium(II) aquo ammine species from the ruthenium(III) prodrug should be favoured in the relatively reducing and hypoxic environment provided by the interior of many tumours. Screening studies on a series of ruthenium complexes show that many complexes which would be thought to function by an activation-by-reduction mechanism do exhibit antitumour activity. In general, ruthenium complexes are an order of magnitude less toxic than cisplatin; however, they must also be administered at higher doses to achieve the same therapeutic effect [174,175]. The high degree of antitumour selectivity hoped for in

pursuing the activation-by-reduction hypothesis has not yet been obtained.

Tissue distribution studies indicate that significant quantities of the metal injected as ammine ruthenium(III) complexes are retained by several tissues. Most troublesome are relatively high levels remaining in the blood, suggesting that binding to proteins occurs even in this aerated environment. A possible reason for this is the high affinity of both ruthenium(II) and ruthenium(III) ammine complexes for RS^- groups which are available on some plasma proteins. Nevertheless, the prototypical complex, $cis-[Ru(III)(NH_3)_4Cl_2]^+$, does exhibit localization in the tumour on a level comparable with that of the most widely used tumour imaging agent ^{67}Ga -citrate. It has been shown that cellular toxicity of $cis-[Ru(III)(NH_3)_4Cl_2]^+$ is considerably higher than that of the corresponding *trans* isomer when tested on cells grown in liquid media [176]. The spectra of samples of $[Ru(III)(NH_3)_5]^{3n+}$ -DNA prepared from normal and heat-denatured DNA show bands in the visible region, suggesting that helical DNA binds ruthenium(III) primarily at N(7) sites on guanine residues, while the single-stranded DNA coordinates the metal additionally at the exocyclic nitrogen atoms of cytosine and adenosine [177]. Since ammine ruthenium ions can coordinate to *exo*-N sites of cytosine and adenine as well as to ring nitrogen atoms, a variety of options for interstrand and intrastrand cross-linking of DNA becomes available.

In the case of cisplatin the discovery of its antitumour properties was based on the observation that the complex induced filamentous growth in strains of *Escherichia coli*. Likewise this microbiological property has been chosen to compare the biological activity of a series of rare platinum metal complexes of ruthenium, osmium, rhodium and iridium, bearing the ligands [178–180] Cl , H_2O and Cl , NH_3 and Cl^- , dimethyl sulphoxide (Me_2SO) and Cl^- , pyridine and Cl^- . Rhodium(III) and ruthenium(III) induce filamentous growth, the rhodium(III) derivatives being the most active. Both the derivatives of ruthenium(II) with Me_2SO and that of rhodium(III) with pyridine induced filamentous growth similar to that using cisplatin. Of all the chloroammine derivatives of ruthenium and rhodium tested, the neutral species *fac*- $[RuCl_3(NH_3)_3]$ [181] and *mer*- $[RhCl_3(NH_3)_3]$ [178] give the best antineoplastic activity. Using imidazole instead of ammonia (a ligand more able than NH_3 to stabilize ruthenium(II) with respect to the ruthenium(III) oxidation state) a ruthenium complex is obtained which is more active than *fac*- $[RuCl_3(NH_3)_3]$ (and therefore also than cisplatin) towards P-388 leukaemia [196]. This anionic complex $[RuCl_4(Im)_2]^- ImH^+$ (Im = imidazole) has octahedral geometry with two imidazole molecules *trans* to each other and the four Cl^- anions on the equatorial plane. Keppler et al. [182] reported a complex, budotitane, exhibiting antitumour activity with less severe side effects.

The ruthenium complex [183,184] *cis*-[Ru(II)Cl₂(Me₂SO)₄] has octahedral geometry with two *cis* chlorine atoms; three Me₂SO molecules are bonded through the sulphur atom in a *facial* configuration, while the fourth is bonded through the oxygen [185]. The complex has an activity similar to that of cisplatin towards both primary tumour growth and the formation of metastases [186]. Reactions of *cis*-[Ru(II)(Me₂SO)₄Cl₂] with DNA and with some of its bases in aqueous solution have been reported [187]. The most likely sites of reaction in double-stranded polynucleotides are N(7) of both guanine and adenine. Contrary to what was found with cisplatin the reaction with *cis*-[Ru(II)(Me₂SO)₄Cl₂] stabilizes the ordered *B* structure of DNA. However, the reaction produces marked cleavage of the chain.

The synthesis, antitumour activity and X-ray structure of another ruthenium complex, a representative of a new class of water-soluble heterocyclic coordinated ruthenium complex with anticancer activity, bis(imidazolium)(imidazole)pentachlororuthenate(III) ((ImH)₂(RuImCl₅)), has been reported [188]. The life span of the animals treated with (ImH)₂(RuImCl₅) increased up to *T/C* values of 150–162%. This effect was in the same range as that observed with the positive 5-fluorouracil and cisplatin controls. These clinically used drugs increased the life span in the same experiment up to *T/C* values of 144% and 175% respectively. Initial results in testing on autochthonous tumour exhibited good activities against colonic tumours.

The effects of [Ru(NH₃)₆]³⁺ on the conformation of poly(dG-m⁵dC) · poly(dG-m⁵dC) (m⁵dC = 5-methyldeoxycytidine) **I**, and poly(dGdC) · poly(dGdC) **II** were studied [189] by CD spectroscopy. [Ru(NH₃)₆]³⁺ at very low concentrations provokes the *Z*-DNA conformation in both polynucleotides. The results demonstrate that [Ru(NH₃)₆]³⁺ is a highly efficient trivalent cation for induction of the *B*-to-*Z* transition in **I** and **II**. In contrast, [Ru(NH₃)₆]³⁺ has no significant effect on the conformation of calf thymus DNA, poly(dAdT) · poly(dAdT) and poly(dAdC) · poly(dGdT).

The interactions of Ru(III)–chloride systems with DNA were studied [190] at various *r* values. Electronic spectra, melting curves and sedimentation experiments indicate that ruthenium(III) is bound mainly to the phosphate moieties of DNA, causing stabilization of the double helix. For small values of *r*, renaturation was observed upon cooling, and there is possible interstrand cross-linking that persists at room temperature. During second heating, the melting temperature decreases, indicating substantial interaction of ruthenium(III) with DNA bases. Ruthenium(III) only interacts with the nitrogen of the bases when DNA is almost denatured and this cross-linking reaction seems to be quite strong.

The reaction of [Ru(II)(NH₃)₅(H₂O)]²⁺ with calf thymus and salmon sperm-DNA has been studied over a wide range of transition metal ion

concentrations [191]. Kinetic studies revealed a biphasic reaction, with an initial fairly rapid coordination of the metal ion being followed by slower reactions. Binding studies showed that the predominant binding site on helical DNA is the major groove at N(7) of guanine. However, steric hindrance prevents coordination to 40% of the G sites. Increased binding to G residues neutralizes the polyanionic charge in some regions and induces DNA unwinding, strand separation and finally precipitation. Unwinding and/or base pair weakening facilitates metal ion attack at the additional coordination positions at A-N(1) for $[\text{Ru(II)(NH}_3)_5]^{2+}$ and A-N(6) and C-N(4) for $[\text{Ru(III)(NH}_3)_5]^{3+}$, with binding of the latter being under catalytic control, dependent upon the oxidation conditions. Autooxidation of the ruthenium(II) ions regenerated by the presence of a reductant results in a significant DNA strand cleavage but this is diminished upon direct coordination of the metal ion to G-N(7) sites in the major groove. Further investigation will be necessary to determine whether (1) coordination of ruthenium(II), (2) coordination of ruthenium(III), (3) metal-ion-induced structural changes in the nucleic acid, (4) metal-catalysed guanine loss and (5) metal-assisted guanine autooxidation, or other factors prevail in the oncological properties of ruthenium ions.

More recent studies [192] have characterized some aspects of the anti-metastatic properties of coordination metal complexes other than platinum compounds. Rhodium(I) and iridium(I) derivatives of the type $[\text{MChel(L-L)}]^{+/-}$, (Chel = pyridinalimine (N-N-R), acetylacetonate; L-L = 1,5-hexadiene, 1,5-cyclooctadiene, norbornadiene) both with square-planar structure and an octahedral ruthenium(II) derivative $\text{RuCl}_2(\text{Me}_2\text{SO})_4$ were tested using the solid metastasizing tumour of the mouse, Lewis lung carcinoma. The conclusions which can be drawn from the resulting data concern the roles of the metal, of the leaving group and also of the non-leaving group. Organometallic complexes of rhodium(I) are more active than those of iridium(I); within the rhodium(I) derivatives of the type $\text{Rh(I)COD(N-N-R)}^+\text{Cl}^-$ (R = CH_3 , C_2H_5 , ^iPr), the higher the hydrosolubility, the higher is the antitumour and particularly the antimetastatic effect. As far as the diolefinic ligands are concerned, the higher the chelating effect, the higher are the antimetastatic properties of the resulting compound. Separate conclusions can be drawn for the ruthenium(II) derivative. A comparison of its antimetastatic effects with those of cisplatin using three solid mouse tumours clearly shows a better therapeutic index for the former, suggesting that within this class of compounds it is conceivable to obtain derivatives with an antineoplastic activity comparable with or even higher than that of cisplatin.

The reaction of $[\text{Ru(II)(NH}_3)_5(\text{H}_2\text{O})](\text{PF}_6)_2$ with bleomycin sulphate gave stable products following oxidation to the ruthenium(III) analogue [193].

Spectroscopic and electrochemical measurements indicated monodentate binding of $[\text{Ru(III)(NH}_3)_5]^{3+}$ to the imidazole and pyrimidine moieties, with coordination to the latter involving the exocyclic amine nitrogen atom. DNA cleavage studies showed the complexes to be ineffective in DNA strand scission. In vitro these adducts were cytotoxic to L1210 murine leukaemia cells.

Ho et al. [194] studied the interactions of ruthenium hexaammine with Z-DNA and the crystal structure of a $[\text{Ru(NH}_3)_6]^{3+}$ salt of d(CGCGCG) has been determined. A crystal of d(CGCGCG) in the Z-DNA lattice was soaked with hexaammine ruthenium(III) (I), and its structure was refined at 1.2 Å resolution. Three unique metal complexes were found adsorbed to each hexamer duplex. In addition, two symmetry-related binding sites were located, yielding a total of five ruthenium complexes. One unique site and its symmetry-related site were nearly identical with the binding site of hexaammine cobalt(III) (II) on Z-DNA. At that position, the metal complex bridged the convex surfaces of two adjacent Z-DNA strands by hydrogen bonds to the N(7) and O(6) functional groups of the guanine bases. The remaining three binding sites were not present in the II-Z-DNA structure. Of these, two were related by symmetry and spanned the gap between the convex outer surface of one Z-DNA strand and the helical groove crevice of a neighbouring strand. The third site I has no symmetry partner and involved interactions only with the deep groove. In this interaction, the metal complex hydrogen-bonded to both the phosphate backbone and to a set of primary shell water molecules that extended the hydrogen-bonding potential of the deep groove crevice out to the surface of the molecule. Solution studies comparing the CD spectra of low-salt poly(dGdC) · polyy(dGdC) samples in the presence of I and II showed that I did stabilize Z-DNA in solution but not as effectively as II. This suggested that some of the interactions available for the larger ruthenium complex may not be important for stabilization of the left-handed DNA conformations.

The activity of a novel ruthenium compound [195,196] $[\text{RuCl}_4(\text{IM})_2]^- \text{ImH}^+$ was compared with that of 5'-deoxyfluorouridine (5'dFU) in autochthonous acetoxymethyl(methylnitrosamine) (AMMN)-induced colorectal cancer in rats. The ruthenium compound had considerable antitumour efficacy compared with that of 5'dFU against the growth of AMMN-induced colorectal adenocarcinoma in rats. The mortality rates with the complex were dose related but its efficacy did not vary in all the doses administered.

In view of these observations, it is reasonable to assess that the use of rare platinum group metal complexes, in particular octahedral ruthenium derivatives, could give rise to drugs remarkably more active and less toxic than cisplatin.

E. BIOLOGICAL CONSEQUENCES OF PLATINUM COORDINATION COMPOUNDS INDICATIVE OF REACTIONS WITH DNA

Cancer cells differ in two fundamental respects from their normal progenitors. Firstly, in cancer cells the genetic control of life span is lost, which results in immortality. Secondly, cancer cells are more or less unresponsive to feedback mechanisms from neighbouring cells, and as a consequence, cell division is no longer modulated by contact inhibition which gives cancer cells the potential of uncontrolled proliferation [197]. Cancer can arise from abnormal expression of certain genes called proto-oncogenes [198]. Proto-oncogenes are normal cellular genes that are present in every cell of the body and are carefully controlled because they seem to be critical for normal cell proliferation and differentiation. There is considerable evidence, mainly from biochemical studies, that DNA is the principal target of *cis*-DDP *in vivo* and that the interactions of *cis* platinum(II) complexes with DNA impair its function as a template for further DNA replication [31].

(i) *Filament formation in bacteria*

When a low alternating current was passed via platinum electrodes to growing gram-negative bacteria, cell division was inhibited and the bacteria grew into long filaments [1]. This gave an indication of the biological mode of action of platinum complexes. Salts of other Group VIIIB metals such as rhodium and ruthenium have been shown to produce filamentous growth in *E. coli*, but in all cases a much higher concentration was needed than with platinum complexes [199]. Filamentous growth in bacteria is almost certainly indicative of the ability of an agent to react with DNA, leading to a selective inhibition of DNA synthesis with no accompanying effects on other biosynthetic pathways (RNA or protein synthesis). The concomitant cellular growth indicates that RNA and protein synthesis are proceeding normally. Other evidence supporting the mechanism for the induction of filamentous growth by the platinum complexes came from tracer studies [200] which compared the distribution of platinum ions within *E. coli*, after the induction of filaments with *cis*-[Pt(NH₃)₂Cl₂] and after growth inhibition by [PtCl₆]²⁻. In the filamentous cells, platinum ions were associated not only with metabolic intermediates but also with cytoplasmic and nucleic acids, whereas in the cells inhibited by [PtCl₆]²⁻ the platinum combined only with cytoplasmic proteins. The accumulation of areas of strikingly enhanced electron density within platinum-induced filaments of *E. coli* (thought [201] to be aggregates of ribonucleoprotein that had lost its usual distribution pattern but had retained some degree of biochemical integrity owing to its unimpeded rate of synthesis) was also therefore consistent with this pro-

posed biochemical mechanism of filamentous formation. Other known DNA-damaging agents such as UV and X-radiation and cytotoxic alkylating agents produce similar effects [31].

(ii) Induction of lysogeny

Further important evidence for direct attack on DNA by platinum complexes was provided by Resolva [202] who investigated the ability of platinum compounds to induce the growth of phage from lysogenic strains of *E. coli* bacteria. The release of the phage DNA to direct synthesis of new phage is normally a rare event. However, agents reacting with DNA can cause the phage DNA to be released and phage particles to be released with consequent cell lysis. There is an excellent correlation between the anti-tumour activity of platinum compounds and their ability to induce lysogenic *E. coli* to enter the lytic cycle [7]. The interactions of platinum complexes with viruses (papova virus, SV40) have further indicated the importance of reactions with DNA compared with those with proteins in producing biological effects [203] (inactivation of the infectious activity).

(iii) Inhibition of DNA synthesis

The major biochemical effect of *cis*-DDP on cells is inhibition of replication. *cis*-DDP selectively and persistently inhibited the rate of DNA synthesis compared with its effects on RNA and protein synthesis in human AV₃ cells in culture [204] and in Ehrlich ascites cells in vivo [205]. These observations were confirmed in HeLa cells in culture and were extended to show that such selective inhibition of DNA synthesis occurs with low doses of the drug, which showed only minimal cytotoxicity as measured by effects on colony forming ability [206–208]. Harder and Rosenberg [204] further showed that those compounds effective against S180 and also causing filament formation displayed similar effects, whereas the inactive compounds showed no effects until very high dose levels were employed. The inhibition of *cis*-DDP appears to result from inactivation of DNA as a template rather than from interference with the enzymes involved in DNA synthesis. Incubation of DNA polymerase with *cis*-DDP results in enzyme inactivation at high doses of platinum [209].

cis-Dichlorodiammineplatinum(II) is a potent mutagen inducing both frame shift [210] and base substitution mutations [211]. *trans*-DDP is considerably less mutagenic than *cis*-DDP even though at equitoxic doses more of the *trans* isomer is bound to DNA. Studies on bacterial [212,213] and human cells [214] show that repair-deficient mutants are more sensitive to *cis*-DDP than their repair-proficient counterparts.

F. ROLE OF CROSS-LINKING REACTIONS

Although emphasis has been placed on the intrastrand cross-link between adjacent bases on the same strand of DNA as the critical DNA lesion produced by cisplatin, interstrand cross-link production may also be important. The structural requirement for bifunctionality and the principal biochemical effects of the platinum compounds suggest a parallel between the platinum drugs and the bifunctional alkylating agents such as nitrogen mustards. The latter compounds have been thought to produce inhibition of DNA synthesis by their ability to produce cross-links in DNA mammalian cells. It has, however, been a matter of contention as to whether the principal lesion is a cross-link between strands of DNA helix or cross-links between bases on one strand of DNA or possibly between DNA and protein [215]. A reinvestigation of cross-linking of DNA by platinum(II) compounds by the alkaline elution method [216] has confirmed the greater ability of *cis*-DDP compared with *trans*-DDP to cross-link cellular DNA. Several sensitive and/or resistant pairs of L1210 cells have been studied by alkaline elution, and the results indicate that interstrand cross-linking alone is not sufficient to account for the sensitivity to the drug [217]. This observation led Strandberg et al. [217] to propose that an adduct not detected by alkaline elution, such as an intrastrand cross-link, must be responsible for the cytotoxicity of *cis*-DDP. A further study employing alkaline elution showed that the cross-linking effect produced by *cis*- and *trans*-DDP could be separated into two components, one proteinase sensitive and due to DNA-protein cross-links, the other proteinase resistant and due to DNA interstrand cross linking [218]. DNA protein cross-links were at maximum levels immediately after drug removal, while DNA-DNA interstrand cross-links reached maximum levels 6–12 h after drug removal. Toxicity of the two agents in L1210 leukaemia cells and in V79 Chinese hamster cells correlated well with DNA interstrand cross-linking but not with DNA-protein cross-linking [218,219]. However, studies of certain mouse leukaemia L1210 lines resistant to *cis*-DDP [220,221], as well as studies of Walker carcinoma cells [222], have indicated that there is not always a simple correlation between interstrand cross-link formation and cell kill. The biological relevance of DNA-protein cross-links remains unclear. They comprise only a very small fraction (0.15%) of the total Pt-DNA adducts formed in vivo [223].

The effect of specific types of platinum interactions on the stability of DNA was investigated by using three types of DNA-Pt complex representative of different modes of platinum binding [224]. The DNA destabilization action induced with the *cis*-binding complex, cisplatin, can be explained by the rupture of the hydrogen bonding between complementary bases, while

the stabilization of DNA induced with the *trans* compound is probably due to platinum binding between a base (G-N(7)) and phosphate groups in DNA. The decrease in viscosity of DNA is significantly greater for the *cis* than for the *trans* compound and is considered to be a reflection of the shortening of the DNA molecule. This was shown not to be due to the introduction of chain breaks in the DNA. Monodentate binding induced by [Pt(dien)Cl]Cl had no effect on viscosity.

An interesting speculation on the possible mode of binding of Pt-DNA comes from studies of cell killing and mutation induction by *cis*-DDP and *trans*-DDP in *E. coli* cells with different repair capacities [225]. Nonsense mutants resulting from base pair substitution are only produced in wild-type cells with cisplatin, suggesting that the intact products of both the *uvrB* and *recA* gene are necessary for the repair responsible for this type of mutagenesis. Investigation of the nonsense mutants revealed that 70% of these mutations result from GC → TA or GC → AT substitutions at sites where guanine is part of a GAG or GCG sequence. Conceivable intrastrand cross-links between two guanines separated by a third base are responsible for the base pair substitution mutagenesis. Such a reaction is only possible if the base between the guanines becomes unstacked and a microloop is formed in the DNA, a conformational change which could account for the observed platinum-induced physiochemical changes in DNA.

G. ROLE OF DNA REPAIR

The consequences of damage to DNA of living cells must result from three related factors: (1) the number of damaged sites, (2) the ability of the cell to repair the damage, and (3) the temporal relationship between damage production, damage repair and the normal cell functions with which unrepaired damage could interfere. The scheme [226] shown in Fig. 9 suggests an equilibrium between DNA damage and repair superimposed upon ongoing DNA functions such as transcription and translation. The state of the equilibrium at the time and at the site at which a normal DNA function is to occur could determine the consequences of DNA damage. Therefore dif-

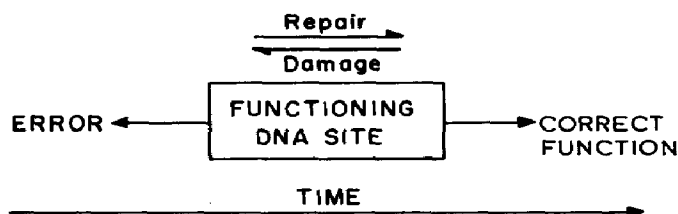


Fig. 9. A schematic representation of the interaction between DNA damage and repair of a random functioning DNA site.

ferences between the susceptibility of cell types to DNA-damaging agents may result from differences in the initial frequency of DNA damage, differences in the capacity of the cell to repair damage or differences in the ability of the cell to tolerate damage either through bypassing damage or abating normal function until damage is repaired.

A positive indication of the role of excision repair of DNA damage in facilitating recovery from cisplatin-induced cell killing has come from studies of DNA damage and cell survival in stationary culture of Chinese hamster [227] or human foetal lung cells [228]. It was found that during post-treatment incubation of the stationary-phase cells, platinum was slowly excised from their DNAs and their plating efficiencies slowly increased. There was a similar relationship between the amount of platinum bound to DNA and cytotoxicity. DNA interstrand and DNA-protein cross-links were also lost from human cells during the recovery period at a rate comparable to that of the loss of total DNA-bound platinum. These results strongly support the hypothesis that damage present on the DNA template at the time of entry of cells into the proliferative cycle was responsible for cellular toxicity and that the repair process was actually effective in achieving biological recovery.

The cells can differ in their abilities to recover from cisplatin-induced toxic damage. This is indicated from studies on LoVo cells [229]. These cells were clearly unable to recover from either potentially lethal or sublethal damage induced by the drug. Consistent with this observation was the similar sensitivity of stationary-phase and exponentially growing LoVo cells. It is speculated from the work done in mammalian cells [230] and on the basis of X-ray crystallographic data [91] that a cisplatin-DNA cross-link is not effectively recognized or repaired.

Another study identified other bacterial DNA repair pathways: the mismatch repair pathway and the adenine methylase activity [231]. The former pathway corrects mismatched DNA base pairs at the replication fork. Bacteria deficient in adenine methylase activity, the latter pathway, were hypersensitive to cisplatin, probably because of decreased Pt-DNA adduct removal. Insertion of a genetic mutation to abrogate mismatch repair, abrogated the hypersensitivity of the bacteria to cisplatin. Cisplatin-DNA adducts trigger several genetically distinct DNA repair pathways [232]. The efficient interaction of these pathways may be required for cell survival following cisplatin-DNA damage.

H. IMMUNOCHEMICAL STUDIES

The suggestion by Rosenberg [233] that platinum compounds may interact with cells so as to modify their antigenic properties by exposing new

antigenic sites on the cell surface has stimulated studies of changes in the agglutinability of tumour and normal cells with various lectins [234] and of the changes in their surface ultrastructure induced by cisplatin [235]. These studies indicated a correlation between the cisplatin-induced release of mucopolysaccharides (sialic acid) from the cell surface and the decrease in cell agglutination with canavalin A and wheatgerm agglutinin. Kleinerman et al. [236] have shown that treatment of human monocytes with cisplatin in vivo results in an enhanced spontaneous cytotoxicity towards target red cells. Moreover, cells taken from patients undergoing cisplatin chemotherapy exhibit a similar effect [237]. Immunostimulatory or immunosuppressive effects were produced by iproplatin as well as by cisplatin in a mixed lymphocyte tumour cell culture system, depending on the ratio of immunocyte responder to tumour stimulatory cells [238]. Cisplatin-induced immune responses in some studies were reported [239,240]. In addition monocyte chemotaxis has been found to be inhibited by cisplatin [241].

When DNA containing bound *cis*-DDP is injected into animals, antibodies are raised which can be isolated and used as tools for studying Pt-DNA interactions. Immunologic techniques have led to a new understanding of the critical determinants of cisplatin's cytotoxic potency. Sundquist et al. [242] examined calf thymus DNA treated with cisplatin or the inactive analogue *trans*-DDP and chloro(diethylene triamine)platinum(II)chloride, [Pt(dien)Cl]Cl, using antinucleoside antibodies. The antibodies react strongly with nucleosides in denatured DNA but not with nucleosides in a Watson-Crick duplex. Although cisplatin was capable of generating antibody-reactive species at high levels of DNA modification, it was *trans*-DDP that generated more antibody binding sites at low levels of DNA modification. The monofunction binding of [Pt(dien)Cl]Cl to DNA did not affect DNA reactivity with the antibodies, indicating that bifunctional DNA binding was necessary to disrupt helical base pairing sufficiently to create an antibody-reactive DNA species.

Cisplatin and *trans*-DDP have different potencies for exposing antibody-reactive nucleosides in naturally occurring DNA and each drug exposes different nucleosides. *trans*-DDP-modified DNA reacted with antibodies to all four nucleosides, while cisplatin-modified DNA reacted with the following: anticytosine > antiadenosine $\approx$ antithymidine $\gg$ antiguanosine. This is consistent with the belief that the major cisplatin adduct is the d(GpG) intrastrand cross-link. Relatively lower levels of DNA modification caused by *trans*-DDP produced large DNA disruptions, which may make these modifications readily recognizable by cellular DNA repair systems. The more covert nature of cisplatin-DNA modifications, especially at adjacent guanines, may prevent this adduct from being repaired in cells. Thus cisplatin may be more disruptive to DNA-dependent cellular functions.

Foka and Paoletti studied the cisplatin–chromatin interactions [243]. They provide evidence that linker DNA may be the preferential target of cisplatin in chromatin and that the DNA itself, rather than chromatin proteins, is the actual drug-binding site. However, as the drug concentrations rise, histones, like the underlying DNA, may also become a source of chromatin-bound cisplatin.

A long-sought goal of cancer pharmacological research is a rapid method of *ex vivo* determination of *in vivo* tumour sensitivity. A novel means of growing explanted human tumours on a cell-adhesive matrix has been developed. Using this system, Tofilon et al. [244] have attempted to quantify sister chromatid exchange (SCE) induction by cisplatin in explanted human tumours. Interestingly, cisplatin's response was heterogeneous, as primary cultures contained cells both sensitive and resistant to cisplatin. Reasonable agreement was noted between the SCE induction and cytotoxicity produced by cisplatin. Both this new cell culture system and the results that have been generated with it may add to our understanding of human tumour biology and antineoplastic drug effects at the cellular level.

Cisplatin can produce chromosomal breakage (clastogenicity) as well as SCE. However, the potency of this clastogenicity is markedly increased in the lymphocytes of patients with Fanconi's anaemia, an autosomal recessive disorder in which the frequency of spontaneous clastogenicity is high. Poll et al. [245] postulated that cisplatin-induced clastogenicity was produced by DNA intrastrand cross-links, whereas *trans*-DDP was a poor inducer of clastogenic effects in Fanconi's anaemia lymphocytes.

Two recent studies [246,247] on the mechanism of resistance (to cisplatin treatment) have focused attention on the cell membrane as a potentially important target of cisplatin's cytocidal actions and, concomitantly, as a potential source of biochemical modulation of sensitivity or resistance to cisplatin. In the murine leukaemia L1210 model system, cisplatin's resistance was associated with marked alterations in amino acid–membrane transport systems and metabolism. In human K562 cells resistant to cisplatin, characteristics of neutral amino acid transport systems differed from those in the cisplatin-sensitive parent line. Two human prostate tumours with different sensitivities to cisplatin were studied by Metcalfe et al. [248]. Small differences were found in the sulphhydryl content of each line, but these were not thought to be the aetiology of the difference in cisplatin sensitivity. However, the more sensitive line took up more ^{191}Pt -labelled cisplatin and this increase was mirrored by an elevated binding of ^{191}Pt to cellular DNA and protein. Differences between the cell lines in metallothionein content might also have contributed to cisplatin resistance. These studies in cisplatin-resistant lines focus on membrane-mediated processes as critical determinants of the antineoplastic actions of cisplatin. The nucleus and repair of its

cisplatin-damaged DNA is clearly not the only subcellular compartment worthy of investigation as an important site of cisplatin's cytotoxic actions or cisplatin resistance.

Murine anticolon antibodies [249] that bound selectively to adducts formed on DNA by the antitumour drug *cis*-DDP or to the inactive isomer *trans*-DDP were elicited by immunization with calf thymus DNA modified with either *cis*- or *trans*-DDP at ratios of bound platinum per nucleotide $(D/N)_b$ of 0.06–0.08. The binding of two monoclonal antibodies to *cis*-DDP-modified DNA was competitively inhibited (50% control) in an enzyme-linked immunosorbent assay (ELISA) by 4–6 nM concentrations of *cis*-DDP bound to DNA $((D/N)_b = 0.031)$. Similar concentrations (4–6 nM) of *cis*-DDP-modified poly(dG)·poly(dC) also inhibited antibody binding, whereas higher concentrations (17–36 nM) of *cis*-DDP-modified poly(dAG)·poly(dTC) were required for inhibition. Adducts formed on other synthetic DNA polymers did not inhibit antibody binding to *cis*-DDP–DNA binding. The biologically active compounds, Pt(en)Cl₂, Pt(1,2-diaminocyclohexane)Cl₂ and carboplatin, all formed antibody-detectable adducts on DNA, whereas the inactive platinum complexes, *trans*-DDP and [Pt(dien)Cl]Cl did not. The monoclonal antibodies therefore recognize a bifunctional Pt–DNA adduct with *cis* stereochemistry, in which platinum is coordinated by two adjacent guanines or to a lesser degree by adjacent adenine and guanine. A monoclonal antibody raised against *trans*-DDP–DNA was competitively inhibited in an ELISA by 40 nM *trans*-DDP bound to DNA, $(D/N)_b = 0.022$. This antibody cross-reacted with unmodified denatured DNA. Its binding to *trans*-DDP–DNA was selectively inhibited by *trans*-DDP-modified poly(dGT)·poly(dCA) (50% inhibition at 1 nM bound *trans*-DDP). The recognition of *cis*- or *trans*-DDP-modified DNAs by monoclonal antibodies thus parallels the known modes of DNA binding of these compounds and may correlate with their biological activities.

Three murine leukaemia lines resistant to cisplatin and one line resistant to dach-Pt(II) complexes were compared with their platinum-sensitive parent lines to determine whether differences in net platinum accumulation were related to the resistant phenotype [250]. The cisplatin-resistant lines (L1210 Pt R4, L1210 DDP5, P388 Pt R4) and the dach-resistant line (L1210 dach) were incubated in vitro with cisplatin, [Sp-4-2-(1*R*,2*R*)-(1,2-cyclohexanediamine-*N,N'*)dichloroplatinum(II)] or carboplatin and the time-dependent cellular platinum levels were determined by flameless atomic absorption spectroscopy. Cell lines resistant to a given platinum complex showed reduction in the rate of platinum accumulation when compared with the sensitive line at 37°C. The data suggest that the mechanism of platinum resistance in these cell lines may be related to a reduced accumulation of the

platinum-containing drug, although patterns of cross-resistance suggest other mechanisms may also be operative.

Inherent sensitivity of cultured human embryonal carcinoma cells to adducts of *cis*-DDP on DNA was reported by Roberts and coworkers [251]. Measurement of survival and DNA binding following treatment of human and mouse embryonal carcinoma cells with cisplatin showed that at a given level of platinum binding to DNA, far greater levels of cell killing were produced than in most other cell types, indicating that these embryonic carcinoma cells are extremely sensitive to DNA damage induced by cisplatin. Cross-linking of glutathione to DNA by cancer chemotherapeutic platinum coordination complexes was reported by Eastman [252]. Glutathione can modulate the toxicity of various drugs, although its role in modulating toxicity by anticancer drugs is ambivalent. At physiological concentrations, glutathione can inhibit the reaction between DNA and *cis*-dichloro(ethylenediamine)platinum(II). Glutathione can also react with monofunctional adducts in DNA to produce a glutathione-Pt-deoxyguanosine cross-link which would reduce the potential toxicity of the drug. The relative importance of these two mechanisms of detoxification is unknown, although both mechanisms probably contribute to glutathione-modulating platinum toxicity.

Studies on differential repair of Pt-DNA adducts in human bladder and testicular tumour cell lines were reported by Bedford et al. [253]. The formation and removal of four Pt-DNA adducts were immunochemically quantified in cultured cells derived from a human bladder carcinoma cell line (RT112) and from two lines derived from germ cell tumours of testis (833 K and SUSA), following exposure in vitro to $16.7 \mu\text{M}$ ($5 \mu\text{g ml}^{-1}$) cisplatin. RT112 cells were least sensitive to the drug and were proficient in the repair of all four adducts, whereas SUSA cells which were fivefold more sensitive, were deficient in the repair of DNA-DNA intrastrand cross-links in the sequences pApG and pGpG. Despite expressing a sensitivity similar to that of SUSA cells, 833 K cells were proficient in the repair of all four adducts, although less so than the RT112 bladder tumour cells. In addition, SUSA cells were unable to repair DNA-DNA interstrand cross-links whereas 50–85% of these lesions had been removed in RT112 and 833 K cells 24 h following drug exposure. It is possible that the inability of SUSA cells to repair platinated DNA may account for their hypersensitivity to cisplatin.

The effects of coordination of *cis*- and *trans*-DDP with DNA have been measured [254] with regard to DNA synthesis, 3′–5′-exonuclease (proof reading) and 5′–3′-exonuclease (repair) activities of *E. coli* DNA polymerase I. Both isomers inhibit DNA synthetic activity of the polymerase through an increase in K_m values and a decrease in V_{max} values for platinated DNA but not for the nucleoside 5′-triphosphates as the varied

substrates. The inhibition is a consequence of lowered binding affinity between platinated DNA and DNA polymerase and of platination-induced separation of template and primer strands. Strand separation enhances initial rates of 3'-5'-excision of [^3H] dCMP from platinated DNA (proof reading), while total excision levels of nucleotides are reduced. In contrast to proof reading activity, the 5'-3'-exonuclease activity (repair) discriminates between DNA which has reacted with *cis*- and with *trans*-DDP. While both initial rates and total excision are inhibited for the *cis* isomer, they are almost unaffected for the *trans* isomer. This differential effect could explain why bacterial growth inhibition requires a much higher concentration of *trans*-DDP than of *cis*-DDP.

The effects of cisplatin and PtCl_4 on growth, RNA, ribosome and DNA synthesis were also tested [255] in growing yeast cells. Treatment with both compounds caused comparable reduction but not significant delay in the course of growth and RNA synthesis, as well as in DNA and ribosome synthesis rates. These observations indicate that PtCl_4 and cisplatin caused defects in the nuclear DNA. Differences existed in the nature of the DNA damage.

Although the well-characterized antineoplastic activity of *cis*-DDP most probably results from reactions with DNA, *cis*-DDP readily inactivates many proteins in vitro. In nearly every reported study, protein reaction with *cis*-DDP is mediated by selective binding to the side-chain of methionine or cysteine with the formation of highly stable linkages [256,257]. Specific high-affinity reactive sites for *cis*-DDP have been identified for the plasma proteins albumin and α_2 -macroglobulin. Gonias et al. [258] examined in detail the reaction of α_1 -antitrypsin ($\alpha_1\text{AT}$) with *cis*-DDP. Methionine 358 in the plasma protein $\alpha_1\text{AT}$ is an oxidation-sensitive reactive-centre residue critical for proteinase-inhibitory activity. Reaction of $\alpha_1\text{AT}$ with 20 μM to 1.67 mM *cis*-DDP or *trans*-DDP afforded concentration-dependent loss of trypsin-inhibitory activity. Binding assays showed covalent incorporation of 1 mol of *cis*-DDP into each mole of $\alpha_1\text{AT}$. *cis*-DDP protected a single methionine residue from oxidation and made $\alpha_1\text{AT}$ resistant to degradation by papain, which cleaves $\alpha_1\text{AT}$ at met [258]. These observations strongly suggest that *cis*-DDP inactivates $\alpha_1\text{AT}$ by binding exclusively to its reactive-centre methionine. $\alpha_1\text{AT}$ bound twice as much platinum when reacted with *trans*-DDP. The *trans* isomer apparently binds to both the reactive-centre methionine and to the single cysteine residue of $\alpha_1\text{AT}$. Because of its greater selectivity, *cis*-DDP is the superior reagent for modification of $\alpha_1\text{AT}$ reactive-centre methionine.

I. CONCLUDING REMARKS

Apparently great strides in our understanding of the mechanism of action of cisplatin and analogous platinum anticancer drugs have been made

during the past 5 years. Selective inhibition of DNA synthesis appears to be the most likely biochemical lesion leading to cell death. The ability of the cell to replicate its DNA on a damaged template may determine its sensitivity to the drug as well as its excision repair process. Mechanisms relating to tumour cell sensitivity and cellular resistance to a specific DNA repair process are not yet clear. After administration of the drug into the body, apart from the binding of the platinum compound to DNA, many other chemical and biochemical events can take place, such as reactions with blood proteins and peptides. An increased understanding of the molecular basis of these reactions and their biochemical relevance will help us design a new platinum drug with improved activity and less toxicity compared with cisplatin.

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