BioMetals 1992, **5**, 195–208

MINI-REVIEW

From cisplatin to artificial nucleases — the role of metal ion–nucleic acid interactions in biology

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Received 21 July 1992; accepted for publication 10 August 1992

Metal ions and metal coordination compounds bind to nucleic acids in a variety of ways, ranging from weak electrostatic interactions via hydrogen bonding and/or van der Waals forces to strong covalent binding. Metal ions naturally take part in the formation and the degradation of nucleic acids, and the propensity of certain metal coordination compounds to bind to nucleic acids, notably DNA, is exploited in cancer chemotherapy. Moreover, metal compounds have a wide potential as chemical probes for nucleic acid structures and as tools for nucleic acid processing.

Keywords: chemical probes, metal compounds, metal–nucleic acid interactions, platinum antitumor drugs

Introduction and scope

A seemingly unrelated experiment on the effects of an electric field on the cell division of *Escherichia coli* led to the discovery of the antineoplastic activity of *cis*-$(NH_3)_2PtCl_2$ (cisplatin, *cis*-DDP) some 25 years ago. There is convincing evidence now that cisplatin and related platinum compounds bind to DNA and interfere with its replication, even though details of the biological steps of tumor cell death are still poorly understood. This paper briefly reviews present knowledge on the mode of action of platinum antitumor compounds as far as interactions with nucleic acids are concerned, and highlights more recent developments in molecular biology to use metal coordination compounds as probes for studying nucleic acid structure and function.

Antitumor activity of platinum coordination compounds

Since the initial report on the induction of filamentous growth in *E. coli* by certain platinum coordination compounds in 1965, and the discovery of their antitumor activity shortly thereafter (Rosenberg *et al.* 1969), cisplatin has become a major, probably even *the* leading antitumor drug (Figure 1). Cisplatin is presently the drug of choice for the treatment of testicular cancer, and is also used in combination therapy for tumors of the ovaries, cervix, bladder, head and neck, with promising results also on a series of other cancers (for a recent compilation of clinical and chemical aspects of platinum-based chemotherapy, see Howell 1991). The present significance of cisplatin as a chemotherapeutic agent may shortly be outweighed by a second generation platinum drug, *cis*-diammine(1,1-cyclobutanedicarboxylato)platinum(II) (carboplatin, paraplatin) which appears to be superior to cisplatin for its fewer adverse side effects rather than a broader spectrum of activity. On the basis of a few empirical 'rules of thumb', virtually thousands of platinum coordination compounds have been prepared and tested for antitumor activity over the past 20 years. The yield of compounds with activity in one or more experimental systems has been remarkably high (10–20%), certainly much higher than typically found in screening tests of fully organic compounds. Activity of platinum compounds is frequently observed when the following criteria are met:

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(i) The compounds are neutral.
(ii) The compounds are square-planar (Pt(II)) or octahedral (Pt(IV)).
(iii) The compounds have *two cis*-arranged or *one* bidentate leaving group(s).
(iv) The leaving groups have favorable exchange kinetics ('window of lability').
(v) The non-leaving groups, frequently amines, have (at least) one hydrogen at the coordinating nitrogen.

There is, however, a growing list of platinum compounds that obviously violate one or more of these 'rules' but still possess marked activity in certain tumor systems. Examples include cationic platinum complexes (e.g. 'platinum pyrimidine blues'), compounds with platinum in the anion (e.g. $K[Pt(NH_3)Cl_3]$), positively charged triamine compounds (e.g. *cis*-$[(NH_3)_2PtLCl]^+$ with L = cytosine or substituted pyridines; Hollis *et al.* 1989), and even platinum compounds having a *trans* geometry and at the same time lacking the NH proton (e.g. *trans*-$Pt(pyridine)_2Cl_2$; Farrell *et al.* 1989). It is very likely that different mechanisms of action are operative for the structurally difficult compounds.

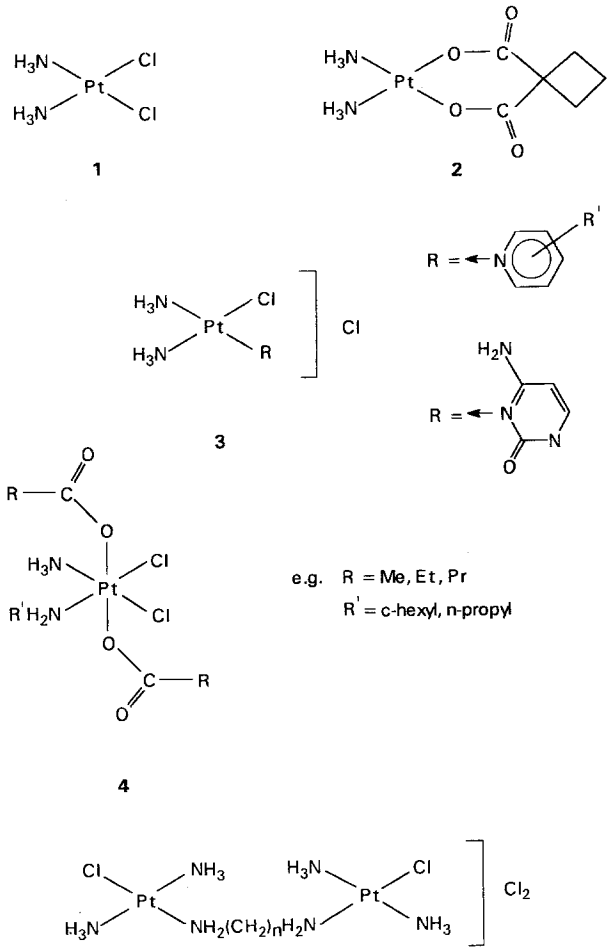


Figure 1. Examples for antitumor-active platinum coordination compounds: (**1**) cisplatin, (**2**) diammine(1,1-cyclobutanedicarboxylato)-Pt(II) (carboplatin, paraplatin), (**3**) *cis*-diamminemonochloro(heterocycle)Pt chloride, (**4**) *cis, trans*-dichlorodicarboxylatomonoamminemonoaminePt(IV), (**5**) μ-diamine-bis(*trans*-diamminechloroPt (II))dichloride.

Among the more recent, promising developments in novel antitumor platinum drugs, only two examples shall be mentioned. (i) Orally active Pt(IV) compounds (Giandomenico *et al.* 1991). These compounds have the common *cis*-$Cl_2(amine)_2Pt$ entity, albeit with two different amines, and in addition have two carboxylate ligands *trans* to each other (Figure 1). It is probably a combination of kinetic inertness (due to the Pt(IV) oxidation state) and favorable solubility and/or distribution (due to their lipophilicity) that is responsible for the effectiveness of these compounds. Intracellular reduction to a Pt(II) species most likely precedes the actual cytotoxic event. Studies that combine cisplatin and these novel Pt(IV) dicarboxylates in an attempt to circumvent cisplatin resistance are of particular interest in this context. (ii) Novel bis(platinum(II)) compounds. These compounds possess the ability to bind to a target molecule, e.g. DNA, at two sites remote from each other. They appear to be of substantial interest both for their potential clinical use and for mechanistic aspects (Farrell *et al.* 1990). Again, activity in cisplatin-resistant cell lines is to be noted.

Basic chemistry of platinum complexes

Reactions of square-planar Pt(II) complexes with biomolecules are, comparatively speaking, slow, and reactions with octahedral Pt(IV) compounds are even more so. This behavior of kinetic inertness of these species is a consequence of the platinum electronic configuration, d^8 and d^6, respectively. Reactions of *cis*-$(NH_3)_2Cl_2Pt$, to which the following brief discussion is restricted, usually take place via a solvolytic pathway (involving a five-coordinate species as an intermediate); hence substitution of one or eventually both chlorine ligands by solvent (H_2O) molecules, followed by reaction with the biomolecule. Solvolysis of chloride is believed to have just the right kinetics (not too fast and indiscriminate, yet not too slow) to guarantee the particular reactivity of cisplatin. A complete hydrolysis scheme for cisplatin with both rate constants and equilibrium constants for the various steps is now available (Miller *et al.* 1991). The distribution

of the various hydrolysis species, which is a function of pH and Cl^- concentration (see below), can be calculated both for conditions inside and outside of a cell (Lim & Martin 1976). This aspect must not be overemphasized, however, in that binding of biomolecules certainly does occur before the thermodynamic equilibrium between the hydrolyzed species is reached. The high extracellular Cl^- concentration (0.1 mM) and the low intracellular concentration (0.004 mM) avoids excessive extracellular hydrolysis and hence reactivity in favor of intracellular reactions. However, it is to be noted that direct nucleophilic substitution of Cl^-, e.g. with thiolate ligands, is also possible in principle.

The hydrolysis scheme of cisplatin is complicated by the possibilty of *cis*-$[Pt(NH_3)_2(H_2O)(OH)]^+$ undergoing condensation reactions to di-, tri- and tetranuclear μ-OH species. Once formed, these species are relatively inert and unreactive, but appear to exhibit considerable neurotoxicity. While in dilute solution their formation is minimal, they could feasibly form upon uneven tissue distribution in areas of high platinum concentration.

The two ammonia ligands of cisplatin are generally believed to be non-leaving groups, which are retained during reactions with biomolecules. This assumption is justified in cases where the newly incoming ligands have a weak or only moderate *trans* effect (e.g. nitrogen or oxygen donors) but clearly not when the ligands *trans* to NH_3 have a strong *trans* effect (e.g. sulfur donors). Then the NH_3 ligands may quickly be lost. Surprisingly, even Cl^- can displace NH_3 *trans* to itself, a situation that allows speculations concerning a principle difference between *cis* and *trans* isomers (Lippert *et al.* 1981), and raises the question of NH_3 possibly adding to the cytotoxicity of cisplatin.

Cisplatin and its target(s)

At a very early stage it became evident that DNA is *a* major pharmacological target of cisplatin and related platinum antitumor compounds (reviewed by Roberts *et al.* 1986). Arguments in support of this view include, among others, (i) the induction of filamentous growth in bacteria, (ii) the induction of lysis in lysogenic bacteria, (iii) the inactivation of viruses and bacteriophages, (iv) the persistent inhibition of DNA synthesis, (v) the mutagenicity of platinum compounds, and (vi) the induction of DNA repair enzymes. Differences in sensitivity of DNA repair proficient and deficient bacteria and cells fit into this picture, as do correlations between the disease response of cisplatin-treated patients and the formation of platinum–DNA adducts in peripheral blood cells (Fichtinger-Schepman *et al.* 1990).

Binding of cisplatin to proteins has been linked to toxicity effects of the drug, but is generally not considered to be related to antitumor activity. Proteins nevertheless appear to play a major role in cisplatin biochemistry, i.e. in the context of the development of drug resistance and/or the repair of platinum–DNA adducts. Thiol peptides and proteins such as glutathione and metallothioneins are suspected to be related to cisplatin resistance, as is a recently discovered protein which belongs to the so-called heat shock protein family (Enns *et al.* 1991). Another 'damage recognition protein' which binds to specific cisplatin cross-links (GpG, ApG) has also been described (Pil & Lippard 1992); however, its precise role is still unclear.

Binding of cisplatin to RNAs and tRNAs has not been studied in great detail.

Finally, there is the possibilty of cisplatin binding to small ions or molecules in the cytoplasma, ranging from simple anions to amino acids, small peptides and nucleotides. Virtually nothing is known about the possible effects of such interactions with regard to antitumor activity, but platinated catabolites have been demonstrated to escape enzymatic processing (Müller & Holler 1989).

Considerably more complex than identifying the (likely) target DNA is the elucidation of the biochemical consequences of platinum binding to DNA. While the understanding of the physicochemical properties of platinum adduct formation — distortion of DNA, unwinding of DNA, melting behavior — is well advancing, the crucial question concerning the biology is still mysterious. Is DNA the only target of biological relevance? Is it really (only) the prevention of new DNA synthesis that causes cell death or are there much more sophisticated pathways? What is the damage to mitochondrial DNA? There are indeed scattered reports in the literature that do not seem to support the idea of inhibition of DNA synthesis as the *ultimate* cause for cell killing. A more recent hypothesis (Eastman & Barry 1991) speculates on the 'activation of a genetic program' leading to 'apoptosis' as a consequence of cisplatin binding to DNA. According to this proposal, the process starts with arrest of the cell division cycle in the G_2 phase and involves endonuclease degradation of DNA in its final stage.

Platinum binding to DNA

Numerous *in vitro* studies on cisplatin–DNA interactions (Roberts *et al.* 1986) have provided a rather

detailed picture of the major reactions that take place. According to this work, the most abundant cross-link is that between two adjacent guanine bases on one strand (d(GpG), 50–60%), followed by bifunctional binding to a guanine and an adjacent adenine on the same strand (d(ApG), 20–30%), and binding to two guanines separated by another nucleobase X (d(GpXpG), 10%). Minor adducts are interstrand cross-links between two guanines (< 1%) and DNA–protein cross-links (<1%), with additional adducts not identified as yet (Fichtinger-Shepman *et al.* 1985).

This general picture is subject to modifications in *in vivo* systems, with the duration of treatment and repair processes becoming increasingly important. For example, interstrand cross-links may then constitute a considerably higher fraction (up to 5%) of the total number of cross-links (Bohr *et al.* 1991). It therefore appears to be premature to draw conclusions concerning *the* most important lesion responsible for antitumor activity. Recent findings (Burnouf *et al.* 1990) on the mutagenicity of cisplatin, according to which d(ApG) cross-links are 5 times more mutagenic than the most frequent d(GpG) adduct, further underline this aspect.

Binding of cisplatin to DNA takes place in two steps: initial monofunctional binding, followed by closure to the chelate. Kinetics of the second step are slower by a factor of 2 only (Bancroft *et al.* 1990). The first step has been modelled by using monofunctional platinum species such as $(NH_3)_3$ Pt(II) or (dien)Pt(II). The effect of monodentate Pt(II) binding on DNA stability is not fully understood and some findings are contradictory. This also applies to biological effects, e.g. the antitumor activity of *cis*-$[Pt(NH_3)_2(N\text{-donor})Cl]^+$ with N-donor being a heterocycle such as pyridine or cytosine, yet the inactivity of $[Pt(NH_3)_3Cl]^+$ and $[(dien)PtCl]^+$.

Binding of platinum electrophiles, whether mono- or bifunctional, occurs preferentially at the guanine N^7 position, which is well accessible in the major groove of B-DNA. However, binding does not take place with equal probability at all guanines. Rather, a directing influence of adjacent bases or even base sequences is often seen. This is of particular significance for the d(GpG) clip of cisplatin, which occurs with a much higher frequency than could be expected on statistical reasons. It even appears that multiple d(GpG) sites in DNA can display markedly different reactivities toward cisplatin (Hemminki & Thilly, 1988). The clue to a full understanding of directing effects of adjacent bases or, similarly, the question why d(ApG) is favored over d(GpA) binding by cisplatin, presumably lies in the *initial monofunctional binding* of cisplatin and the particular steric conditions of the transition state(s).

DNA distortion

The effect of specific bidentate intrastrand cross-links on the DNA structure becomes more and more detailed. Our present knowledge comes from:

(i) Model studies, with X-ray crystallography and NMR spectroscopy being of particular value (reviewed by Sherman & Lippard 1987).
(ii) Physico-chemical methods (e.g. circular dichroism and UV spectroscopy, gel electrophoretic mobility, electrochemistry) (Brabec *et al.* 1990).
(iii) Molecular mechanics calculations, favorably in combination with NMR spectroscopy (Hermann *et al.* 1990).

Clearly, (i) and (iii) can be performed at most with small oligonucleotides rather than large DNA molecules. Information is particularly rich when dealing with specifically platinated oligonucleotides, which are conveniently obtained by annealing a platinated and purified oligonucleotide with the complementary strand. Using recombinant techniques, the insertion of a specifically platinated short oligonucleotide in 'real' DNA has been accomplished (reviewed by Bruhn *et al.* 1990). Then the effect of a particular adduct of cisplatin, e.g. d(GpG), on biological parameters such as *in vitro* replication can be measured. Results from molecular mechanics calculations are to be treated with caution, in that frequently different solutions are obtained and meaningful data require a minimal length of the oligonucleotide (10 bp or longer).

The major *structural* changes of DNA and its constituents as a consequence of bidentate intrastrand binding of cisplatin are as follows:

(i) The two bases involved in cisplatin binding become destacked. Dihedral angles between the bases in DNA are between 70° and 90°. In model compounds angles greater than 100° have been observed.
(ii) The helix axis becomes kinked. Values vary between 30° and 70°, depending on the types of cross-links, the oligonucleotide sequence and the method of its determination (molecular mechanics calculations, NMR, gel electrophoresis).
(iii) The helix undergoes unwinding. The degree of unwinding depends on the adduct. For 1,2

intrastrand cross-links the unwinding angle is about 13° for cisplatin.

(iv) The deoxyribose of 5′ base changes its pucker from S to N.

(v) Hydrogen bonding between complementary strands is somewhat altered (molecular mechanics calculations, NMR) and becomes weaker, as reflected by the exchange behavior of the NH protons in water.

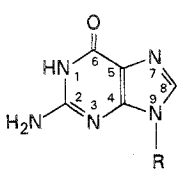
Effects of interstrand adducts, e.g. between two guanines, on DNA structure are just beginning to emerge (Sip *et al.* 1992). With the N^7 positions of two guanines of a $(GC)_2$ sequence being separated by at least 7 Å, the local distortion of DNA might be expected to be considerably larger than that caused by an intrastrand adduct between two adjacent guanines. The complementary cytosines have indeed been shown to be hyper-reactive to hydroxylamine.

Models of platinum–DNA interactions

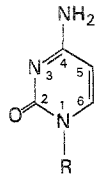
Model nucleobases, nucleosides and nucleotides

In model nucleobases the sugar present in nucleosides and the sugar phosphate entity of the nucleotides are replaced by an alkyl group (Figure 2). Either nucleobase permits the study of platinum reactions with the heterocyclic part of the base; the nucleotide also allows hydrogen bonding interactions (e.g. between amine ligands of platinum) and/or direct metal–phosphate binding ('macrochelate'). As far as we can tell, reactions of platinum at the heterocyclic rings are adequately reproduced even with model nucleobases. This refers in particular to platinum binding sites, conditions of metal binding, and effects on ligand acidity and basicity, dihedral angles in bis(nucleobase) complexes, etc. (for a review, see Lippert 1989). Of the theoretically possible adducts of cisplatin with the four common nucleobases, guanine (G), cytosine (C), adenine (A), thymine (T), or uracil (U), which include four 1:1 and 10 2:1 compounds (different binding sites and 2-fold binding are *not* considered), a fair number of model nucleobase complexes have been prepared and in many cases X-ray structurally characterized. As far as complexes of *cis*-DDP, *trans*-DDP or monofunctional triamineplatinum(II) species are concerned, the following binding sites have been established by crystal structure analyses:

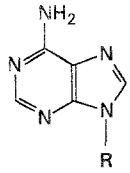
G: N^7; N^1; N^7, N^1; N^7, N^1, N^3
C: N^3
T (U): N^3; N^3, O^4
A: N^7; N^1; N^7, N^1



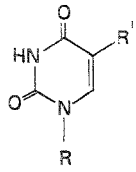
guanine (G)



cytosine (C)



adenine (A)



uracil (R^1 = H) (U)

thymine (R^1 = CH_3) (T)

R = alkyl (CH_3, C_2H_5) : model nucleobase

R = ribose — ribonucleoside
2′-deoxyribose — 2′-deoxyribonucleoside

R = ribose phosphate (3′ or 5′) — ribonucleotide
2′-deoxyribosephosphate (3′ or 5′) — 2′-deoxyribonucleotide

Figure 2. Common nucleobases (major tautomers only) and atom numbering schemes. The RNA base uracil is frequently applied instead of thymine because of its similar coordination chemistry.

Application of other platinum species (Pt(IV), $[Pt(III)]_2$) or combinations of metals (Pt, M) has yielded many more coordination patterns, e.g.:

G: N^7, O^6 (bridging or chelating); N^1, O^6
C: N^3, N^4; N^4; N^3, O^2
T (U): N^3, O^4, O^2
U: C^5

There is good reason to believe that this list is by no means complete. The use of model nucleobases has proven to be advantageous in the synthesis of adducts of yet unknown relevance to the biological chemistry of platinum species, which very well could represent minor adducts. For example, model compounds for potential cross-links in the interior of DNA, involving the N^3 positions of C and T or N^1 of G have been prepared and structurally characterized. Some examples are given in Figure 3.

Oligonucleotides

As outlined above, studies of Pt(II) oligonucleotide interactions have had a major impact on the development of this field. X-ray crystal structures are few

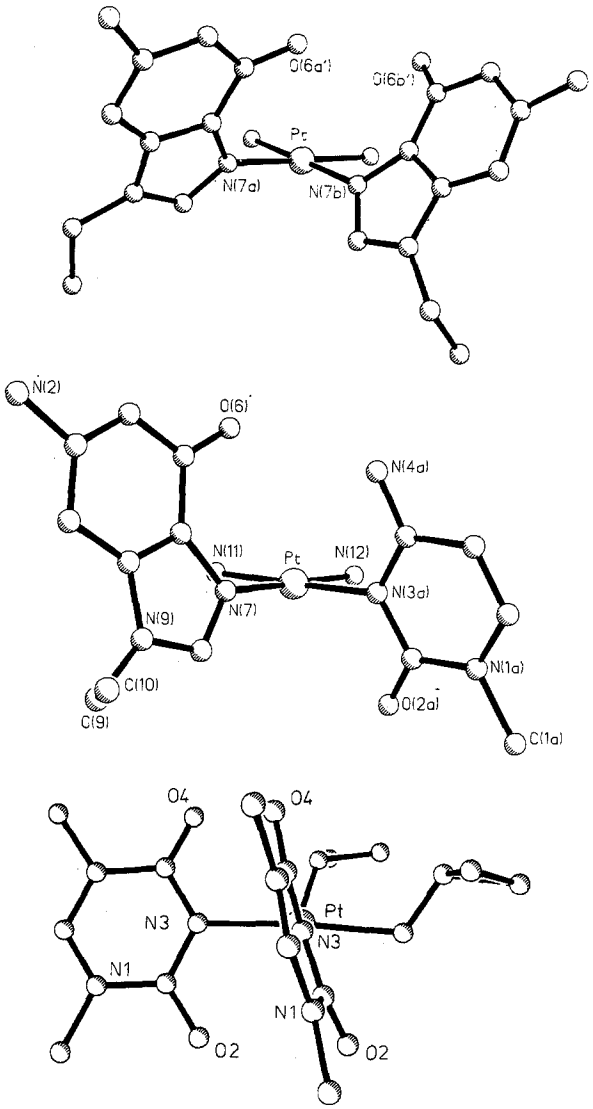


Figure 3. Examples of structurally characterized models of *cis*-$A_2Pt(II)$ cross-links with nucleobases: (**a**) between two N^7 positions of 9-ethylguanine (A = NH_3), (**b**) between N^7 of 9-ethylguanine (deprotonated at N^1) and N^3 of 1-methylcytosine (A = NH_3), (**c**) between two N^3 positions of 1-methylthymine (A = cyclopropylamine). The three compounds represent models of cross-links that occur at the periphery of DNA (major groove) (**a**), between mixed donor sites (exterior and interior of DNA) (**b**) and between two donor sites normally in the interior of DNA (**c**). Note that in all three cases, the two bases adopt a head–head orientation. Dihedral angles between the two bases are 78 ° (**a**), 97° (**b**) and 84° (**c**), but are subject to packing forces (e.g. 68° in (**a**) with a different counter ion).

in number, however. At this point, only three examples are known: *cis*-$(NH_3)_2Pt$([d(pGpG)] (Shermann *et al.* 1985), *cis*-$(NH_3)_2Pt$[d(CpGpG)] (Admiraal *et al.* 1987) and (dien)Pt[d(ApGpA)] (Admiraal *et al.* 1992). (This list does not include soaking experiments with pregrown crystals of tRNAs and a double-stranded B-DNA dodecamer.) In all three compounds, platinum binding is through N^7 of guanine. The solid state structures in all cases are very complex due to the presence of several independent molecules in the unit cell, extensive intermolecular hydrogen bonding and stacking interactions. The basic structural features of GG or GXG cross-linking, as determined by NMR in solution, are generally confirmed in the solid state structures, although there are also differences seen as far as nucleobase stacking interactions are concerned.

Reactions of trans-*DDP*

trans-DDP is the therapeutically inactive isomer of *cis*-DDP. Monofunctional binding of both isomers should be similar, but the bifunctional adducts formed subsequently are markedly different. Both 1,3, 1,4 and longer range cross-links have been detected for *trans*-DDP, yet no 1,2 adducts for obvious steric reasons (Figure 4). The regiospecificity for both isomers is also somewhat different, e.g. with cytosine-N^3 clearly involved in *trans*-DDP cross-links. Whether it is these differences in steric distortion of DNA that could possibly explain the differences in the biology of both isomers, or, as proposed by Ciccarelli *et al.* (1985), differences in repair (with *trans*-$a_2Pt(II)$ removed more efficiently)

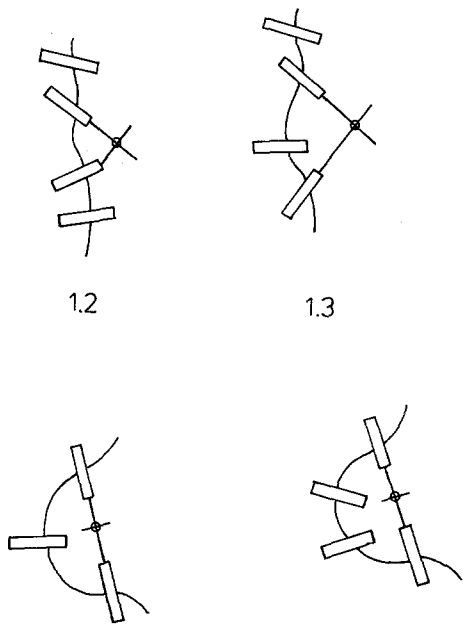


Figure 4. Schematic representation of preferred intrastrand cross-links of *cis*-DDP (top) and *trans*-DDP (bottom).

is not fully clear as yet. A third possibility — differences in the basic chemistry of both isomers — is generally not considered to be valid, even though such differences do exist. One such difference refers to the displacement of a nucleobase L from *trans*-[(a_2Pt(L)Cl]Cl according to

2*trans*-[a_2Pt(L)Cl]Cl →

trans-a_2PtCl_2 + *trans*-[a_2Pt(L)$_2$]Cl_2

(Krizanovic *et al.* 1989). A similar reaction is not expected to occur (and has never been observed) for the *cis* isomer. However, chlorine in *cis*-[a_2Pt(L)Cl]Cl has been shown to labilize a NH_3 in the *trans* position and is capable of expelling it under certain conditions (see above).

Major *trans*-DDP cross-links in single- and double-stranded oligonucleotides have been studied by NMR and molecular dynamics calculations (Lepre *et al.* 1990). Among the most unexpected findings is the observation of a linkage isomerization of a 1,3-bis(guanine) adduct in a single-stranded dodecamer, 5'-d(TCTACGCGTTCT), to a 1,4-guanine, cytosine cross-link (Comess *et al.* 1990).

Metal-modified base pairs and base triples

We have recently started to prepare compounds containing nucleobases covalently linked by a metal of linear coordination geometry, e.g. *trans*-a_2Pt(II), *trans*-a_2Pd(II) or Ag(I). Frequently, the nucleobases still keep some of the hydrogen bonds between each other, thereby generating 'metal-modified base pairs' (Dieter-Wurm *et al.* 1992, Menzer *et al.* 1992). An example of an X-ray structurally characterized compound is shown in Figure 5. Both complementary and non-complementary bases can be linked this way and analogs of the major base-pairing schemes (Watson–Crick, Hoogsteen, base triples) have been synthesized. As has been proposed by us (Dieter-Wurm *et al.* 1992), oligonucleotides suitably modified with *trans*-a_2Pt(II) might be applicable in antisense oligonucleotide chemistry: while the oligonucleotide is responsible for (fast) recognition of the target sequence at either DNA or mRNA, the monofunctionally bound platinum electrophile, in a slower reaction, could irreversibly link the oligonucleotide with the target.

Pt(IV) compounds

It is a widely accepted, albeit not undisputed, opinion that antitumor-active Pt(IV) coordination compounds represent prodrugs that undergo bioreduction to an ultimate reactive Pt(II) species (Eastman 1987, Chaney *et al.* 1991). Interestingly,

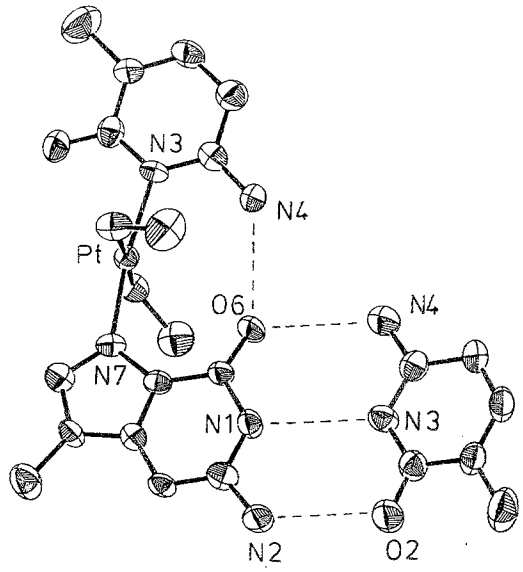


Figure 5. Metal-modified base triple consisting of a Watson–Crick pair between 9-methylguanine and 1-methylcytosine and simultaneously a *trans*-$(CH_3NH_2)_2$Pt(1-methyl-cytosine) entity linked to 9-methylguanine in a Hoogsteen fashion.

however, Pt(IV) drugs and their reduced Pt(II) forms appear *not* to show identical reaction profiles with DNA. Very little systematic work has been done so far on Pt(IV) nucleobase complexes, even though the chemistry is, in some respect, more exciting than that of Pt(II). For example, the long-time disputed guanine-N^7, O^6 chelate of cisplatin has been realized with a 6-oxopurine ligand and $(CH_3)_3$Pt(IV) (Lorberth 1988), and a textbook example of a metal migration process on a nucleobase (1-methylcytosine) involves a Pt(IV) entity (Lippert *et al.* 1986).

Platinum compounds and strong nucleophiles

Reactions of simple platinum amine complexes or their nucleobase adducts with sulfur-containing nucleophiles or even cyanide have received considerable attention in the past both for potential applied aspects and as a tool for biochemists and molecular biologists. Thus diethyldithiocarbamate, thiosulfate and glutathione have been tested as rescue agents that are capable of reducing cisplatin nephrotoxicity while maintaining antitumor activity (for a review, see Lempers & Reedijk 1991). As might have been expected, timing is crucial. The quenching of monofunctional cisplatin–DNA adducts by thiourea or low concentrations of CN^- has been observed, as has been extensive removal of platinum from platinated DNA by excess CN^-. The

basic chemistry underlying these fundamental reactions is not always clear (for platinum–glutathione reactions see, e.g. Appleton *et al.* 1989). Despite the very high thermodynamic stability of a Pt–CN bond, reactions of platinum–nucleobase complexes with CN^- can be remarkably slow (Jones & Beaty 1991) up to the point where the platinum nucleobase become completely inert (Raudaschl-Sieber & Lippert 1985, Frommer & Lippert 1990). The unreactivity is a consequence of a very effective shielding of the platinum by exocyclic groups of the coordinated nucleobases, probably complemented by the inaccessibility of platinum in certain cross-links as a consequence of steric conditions in platinated oligonucleotides or DNA (Schwartz *et al.* 1990).

Modelling base mispairing

The understanding of the molecular basis of metal-caused mutagenicity still represents a great challenge. As far as possible mispairing mechanisms on the template level are concerned, model studies can provide the rationale for such processes. The choice of Pt(II) or Pt(IV) turns out to be of considerable advantage in these studies, even in cases without biorelevance: because of inherently slow kinetics, intermediates may be trapped which normally, with kinetically labile metal species, can neither be observed nor isolated. Among the numerous feasible ways of metal-caused mispairing mechanisms, e.g. effect on tautomer structure (metal-induced shift in tautomer equilibria, metal-stabilized rare tautomers), blocking of hydrogen bonding sites by a metal, ionization of weakly acidic NH protons, steric effects on either the substrate, the template or the polymerase, some have been modelled applying platinum as the metal (Faggiani *et al.* 1981, Lippert *et al.* 1986, 1992, Schöllhorn *et al.* 1989).

Nucleic acids and metals other than platinum

Types of interactions

Nucleic acids are polyanions which require, by virtue of this fact, cations for charge balance. Apart from histone proteins with their positively charged lysine and arginine residues, metal cations (K^+, Na^+, Mg^{2+}, Zn^{2+} (?)) are required for this purpose. The anionic charge of nucleic acids at the same time explains their propensity to react readily with free metal ions or metals in cationic coordination compounds. Ways of interactions between metal species and nucleic acids can be non-covalent and covalent, and include the following possibilities (Figure 6)

(i) Ionic attraction,
(ii) Hydrogen bonding between nucleic acids and ligands of (inert) cations (*cf.* the $B \rightarrow Z$ transition of DNA by $[Co(NH_3)_6]^{3+}$).
(iii) Intercalation of metal complexes containing suitable planar, heteroaromatic ligands (e.g. metal porphyrine complexes or, possibly, *o*-phenanthroline compounds).
(iv) Groove binding (minor or major groove) of ligands carrying a metal, possibly in equilibrium with (iii).
(v) Covalent binding with binding sites being the heterocyclic part of a nucleobase (e.g. platinum), the phosphate oxygens (e.g. magnesium), the sugar oxygens (e.g. copper or osmium) or combinations thereof.

Covalent patterns are governed by a combination of factors such as the metal character (soft or hard), solvolysis state of the metal and availability of donor sites (local structure, sequence specificity). As far as its significance in the 'normal' biology of metal ions is concerned, metal–phosphate oxygen interactions dominate.

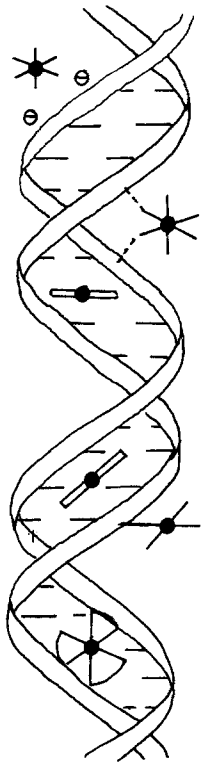


Figure 6. Schematic representation of possible interactions between metal species and DNA (from top to bottom): ionic attraction, hydrogen bonding, intercalation, minor groove binding, covalent binding and major groove binding.

Relevance

Interactions with metal ions are of utmost importance to the chemistry of nucleic acids, both for their biological role (which in many cases is still poorly understood) and their potential applications. Frequently, the metal is at the interface between a nucleic acid and a protein, hence part of a ternary system.

Structural roles of metal ions. Probably the most intensively studied and best understood examples are tRNAs. The specific folding and hence biological activity of these nucleic acids crucially depends on the presence of tightly bound Mg^{2+}. Undoubtedly, metal ion binding is also crucial for the folding of mRNA and unusual DNA structures, e.g. three- or four-way junctions, may also require the presence of metal ions (Guo *et al.* 1990, Lilley 1990).

Effect on duplex stability. Melting and rewinding of DNA is strongly affected by the presence of metal ions (type of metal, concentration). While Mg^{2+} thermally stabilizes DNA, Cu^{2+} at higher r_B values strongly destabilizes duplex DNA. Zn^{2+} is special in that it facilitates both melting and rewinding of DNA, possibly as a consequence of its balanced affinity for hard phosphate oxygens and soft ring nitrogens. Base pairing becomes stronger as the repulsion between negatively-charged polynucleotide strands is diminished by cation binding. When too strong, it may force indiscriminate base pairing, however, and eventually lead to wrong base pair formation. This can occur both at the DNA level or in the mRNA–tRNA interaction during protein synthesis.

Role in catalysis. Both the formation of nucleic acids from mononucleotides and their degradation, the site-specific cleavage of DNA by endonucleases, and nucleoside di- and triphosphate chemistry are closely associated with metal ions (reviewed by Basile & Barton 1989). For nucleases, for example, any of the following functions of metal ions (or combinations thereof) are feasible: (i) substrate (P–O bond) activation as a consequence of metal Lewis acidity, (ii) activation of coordinated H_2O for nucleophilic attack on P, (iii) activation of coordinated H_2O (M–OH formation) for hydrogen abstraction from the sugar 2′ position in RNAs, (iv) structural role for proper orientation of the substrate or stabilization of the 5-coordinate transition state of P, and (v) providing charged phosphate and non-metal catalytic sites, e.g. carboxylates (Jou & Cowan 1991). Required metals are Mg^{2+}, Ca^{2+} and Zn^{2+}, with Co^{2+} and Mn^{2+} also showing activity. Frequently, more than a single metal ion takes part in the catalytic process: The 3′,5′-exonuclease within the Klenow fragment of DNA polymerase I (*Pol*I) from *E. coli* has (at least) two metals in the active site (Beese & Steitz 1989), possibly even three (Han *et al.* 1991), and the restriction endonuclease (*Eco*RI) requires two different metal ions (Mg^{2+} and Zn^{2+}) for activity. These findings are in excellent agreement with conclusions drawn from simple inorganic model compounds (Hendry & Sargeson 1990). There is also strong evidence that RNAs capable of autolytic cleavage at a unique site ('hammer head self-cleaving domain') require divalent metal ions for activity (Dange *et al.* 1990, Dahm & Uhlenbeck 1991).

Redox active metals. The presence of redox active metal species in the vicinity of nucleic acids is dangerous due to the possible formation of radicals, in particular OH·, as a consequence of Fenton and Haber–Weiss chemistry. The anticancer antibiotic bleomycin takes advantage of this fact by binding to DNA and cleaving DNA by a metal ion dependent mechanism (Stubbe & Kozarich 1987, McCall *et al.* 1992). Similar reactions may lead to mutagenesis or even carcinogenesis. For example, it has been proposed that the DNA damage caused by methylhydrazines is a consequence of non-enzymatic activation by redox active metals which produce the ultimate carcinogenic species (Kawanishi & Yamamoto 1991).

Applications of metal compounds in molecular biology

Metal coordination compounds have become a major tool for molecular biologists in recent years (for reviews, see Basile & Barton 1989, Pyle & Barton 1990, Sigman 1990). Applications include both the probing of nucleic acid structures and the use of metal compounds as chemical nucleases.

Metal complexes as probes. Like their purely organic counterparts, metal compounds used as chemical probes utilize hyper-reactivity of certain nucleobases in unusual nucleic acid structures. OsO_4, for example, recognizes exposed thymines present in single-stranded DNA sections or B → Z junctions and covalently adds to the 5,6 double bond of T. The binding sites can be detected by electron microscopy and/or sequencing methods. Guanine bases in non-standard DNA structures (mispaired, bulged,

looped out, terminal) are recognized by certain tetra-aza-macrocyclic nickel complexes. Treatment with the oxidant $KHSO_5$ (conversion to Ni(III)) and subsequent piperidine treatment leads to specific strand scission (Chen *et al.* 1992). Complementary in shape between nucleic acids and coordinatively-saturated metal compounds is another promising approach, pioneered by Barton and co-workers. For example, the Λ and Δ isomers of inert tris(phenanthroline) complexes of Co^{3+} or Ru^{2+} have been demonstrated to interact differently with B-DNA, the Δ isomer being preferred. With left-handed Z-DNA, the situation is reversed in that the Λ isomer binds preferentially (Barton 1986). The chiral discrimination is believed to be the consequence of differences in sterical interactions. Whether the intercalation of one of the *o*-phen wings into DNA is involved is uncertain, considering recent NMR evidence, which favors minor groove binding of both isomers (Eriksson *et al.* 1992). Whatever the exact way of binding may be, a somewhat exotic way of separating enantiomeric metal complexes on an immobilized duplex DNA has already been demonstrated (Baker *et al.* 1991).

Redox active metal compounds. Oxidative attack of metal species in 'high' oxidation states, e.g. oxoruthenium(IV) (Grover & Thorp 1991) and/or radicals on the ribose or deoxyribose entities of nucleic acids causes strand cleavage. Particularly effective in nicking nucleic acids are Cu^+ or Fe^{2+} (generated from Cu^{2+} and Fe^{3+} by suitable reductants such as ascorbic acid or thiols) in the presence of O_2 or H_2O_2. There are many variations of this theme, e.g. the application of bis(1,10 phenanthroline)Cu(I) (Sigman 1990), of methidiumpropyl–EDTA–Fe(II) (Hertzberg & Dervan 1982), or of various metalloporphyrins (Ding *et al.* 1990). There appears to be no unique site of attack on the sugar. Rather, the orientation of the metal that generates the radicals relative to the sugar is of importance (Pratviel *et al.* 1991). The aforementioned compounds are relatively non-specific DNA cleaving agents and therefore ideally suited for footprinting of nucleic acid–protein contacts.

The same applies to the *anionic* $[Fe(EDTA)]^{2-}$ complex which, due to its charge, does not bind to nucleic acids, yet with H_2O_2 generates a flow of OH· onto the nucleic acids (Tullius 1989)

$$[Fe(EDTA)]^{2-} + H_2O_2 \rightarrow [Fe(EDTA)]^{1-} + OH^- + OH\cdot$$

When fixed to a crystal surface (e.g. $Ca_3(PO_4)_2$), the helical periodicity of a particular DNA can be probed. Only those ribose entities that are exposed on the surface are attacked and cleaved by the OH·.

Sequence-specific cleavage of DNA using metal redox chemistry has been achieved by forming conjugates between DNA binding proteins and suitable small ligands with an affinity for transition metals (copper, iron, nickel) (Chen & Sigman 1987, Mack *et al.* 1988, Mack & Dervan 1990) or by suitable modifications of typical DNA binders, e.g. netropsin (Bailly *et al.* 1992). Certainly, *the* most impressive example of selective cleavage of DNA comes from Dervan's group: short pyrimidine oligonucleotides (T, C) equipped with Fe(EDTA) entities at one or both ends bind via triple-helix formation to target sequences on a large DNA molecule and produce, through radical formation, DNA cleavage. Specificity is extremely high: a Fe(EDTA) modified 20-mer has been demonstrated to cut a single site of a 340 kbp chromosome in the presence of 14 million base pairs of a yeast genome (Strobel & Dervan 1990).

Metal-mediated photo-oxidation. The ability of certain transition metal compounds (tris(*o*-phenanthroline) or tris(bipyridine) compounds of Co(III) or Ru(II); Zn(II)-porphyrins; UO_2^{2+}) to produce, upon irridation with light, singlet oxygen ($^1O_2$), which oxidizes nucleic acids, has been successfully applied in footprinting experiments (Nielsen *et al.* 1988) and in characterizing the folded structures of tRNAs (Chow *et al.* 1992).

Hydrolytic cleavage of nucleic acids. It is well established that many cations, both of the main group and transition elements, as well as coordination compounds of Zn(II) or Cu(II), are capable of hydrolyzing RNA. In the case of hydrolytic cleavage of $tRNA^{Phe}$ by Pb(II), a somewhat clearer picture of the catalytic process has emerged, largely based on soaking experiments with tRNA single crystals and X-ray crystallography.

The development of artificial, site-specific nucleases is an area of great current interest (Modak *et al.* 1991). A major advantage of the hydrolytic cleavage of nucleic acids over nucleases based on redox chemistry is the generation of fragments that are viable to subsequent routine enzymatic reactions. Hydrolysis of totally unactivated phosphate diesters such as those in DNA represents a great challenge, especially when binding to a specific site or sequence is required. There have been successful attempts to prepare hybrids between a natural, metal-dependent nuclease (staphylococcal nuclease, Ca^{2+}) and a DNA binding entity (e.g. a repressor

protein or a synthetic oligonucleotide) to ensure site-specific hydrolytic DNA cleavage (Corey *et al.* 1989, Pei & Schultz 1990).

Miscellaneous. There are numerous reports in the literature on other applications of metal ions of metal coordination compounds in DNA chemistry. They include, among others, the use of metals as stains in electron microscopy of nucleic acids or in scanning tunneling microscopy (STM), the preparation of heavy metal derivatives in the X-ray crystallography of nucleic acids and the fractionation of DNAs of different base composition by different metal ions.

Non-platinum metal antitumor compounds

Rosenberg's discovery of the antitumor activity of cisplatin and related platinum coordination compounds has had a truely 'catalytic' effect on the development of anticancer metal compounds (Haiduc & Silvestru 1990, Keppler 1990). Interactions of the active compounds with biomolecules, notably DNA, have been studied, yet not to the extent of platinum complexes. Compounds, for which there is some reliable indication that reactions with nucleic acids are of significance with regard to the mode of action, include the following:

(i) Ruthenium complexes. These are rather versatile in their oxidation state (II and III), charge (cationic, neutral, anionic) and ligands (amines, N-heterocycles, halogens, S-donors), and there is the possibility that they possess no universal mode of action.

(ii) Dirhodium(II)tetracarboxylates. They have been found to preferentially react with adenine nucleobases via the axial positions of the dimetal core and the X-ray structure of a model compound with 1-methyladenosine has recently been described (Rubin *et al.* 1991).

(iii) Metallocene dichlorides $(Cp)_2MX_2$ with M = Ti, V, Nb, Mo. Recent analytical evidence for titanocene binding to DNA (McLaughlin *et al.* 1990) is in support of the proposed interference of these compounds with DNA biosynthesis (Köpf-Maier & Köpf 1988), but their chemistry, as determined for M = Mo(II) in more detail (Kuo *et al.* 1991), strongly rules against a mechanism similar to that of cisplatin.

Many of the other effects of metal complexes on biological systems (metal antibiotics, radiosensitizers, and antiviral, antibacterial and antiparasitic metal compounds) may be related to interactions with nucleic acids, but the evidence in most cases is fragmentary (for a review, see Farrell 1989).

Outlook

The development and study of metal coordination compounds with an affinity for nucleic acids is a rapidly growing, interdisciplinary field. Major applications of such metal complexes are in the areas of medicine and molecular biology. The use of DNA-binding platinum compounds such as cisplatin in the treatment of certain malignant diseases has become routine and metal compounds are becoming indispensable tools for studying the polymorphism of nucleic acids. Many of these applications are still poorly understood as far as the mechanistic aspects are concerned. Knowledge of the basic chemistry of these metal compounds *and* of the target molecule(s) is required to come up with a comprehensive picture.

Acknowledgment

The author wishes to thank M. Gatz, Dr G. Trötscher and M. Lutterbeck for the preparation of the manuscript, and Dr O. Renn for helpful comments.

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