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FEATURE ARTICLE

Improved understanding in platinum antitumour chemistry

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After the serendipitous rediscovery of cisplatin {*cis*-$[PtCl_2(NH_3)_2]$, first synthesized in 1845} in 1964 by Rosenberg, and its routine use as a successful cytostatic drug since 1979, exciting chemistry has resulted from several laboratories, using this simple compound and a variety of its derivatives. The major chemical features that have led to a significant improvement of our insight into the mechanism of action are discussed in this feature article. Key elements appear to be: (*i*) controlled hydrolysis, transport and binding to DNA; (*ii*) a specific binding at neighbouring guanine bases; and in particular (*iii*) a specific distortion of DNA, changing its interactions with proteins.

Introduction

The compound *cis*-$[PtCl_2(NH_3)_2]$, clinically known as 'cisplatin', first described 150 years ago, but in 1964 and 1969 found to be active as a cytostatic, is a very successful antitumour drug.[1] Cisplatin is routinely used for the treatment of testicular and ovarian cancer, and is increasingly used against cervical, bladder and head/neck tumours;[2] typical doses administered to patients are 100 mg/day for up to five consecutive days. A key reaction step in the mechanism of action appears to be the binding of the *cis*-$Pt(NH_3)_2$ unit to cellular DNA at two neighbouring guanine bases, and more specifically at their N^7 atoms.[3]

Derivatives belonging to the *cis*-$[PtX_2(amine)_2]$ structural class (X = leaving anionic group; amine = any primary or secondary amine), show similar or improved biological activity, although up to now only about 10–15 platinum compounds have entered into clinical trials. Similar compounds from other group 10 elements (Ni, Pd) do not yield active compounds; in fact a key factor that might explain why Pt is most useful comes from the ligand-exchange kinetics, which for this type of ligand are of the order of a few hours, thereby preventing rapid equilibration reactions.[1,3] A few compounds of other metals, such as ruthenium, titanium and tin, have been reported to be active and some have just started clinical trials; these will not be discussed here.

Toxic side effects of cisplatin (*e.g.* nausea, ear damage, vomiting, loss of sensation in hands, kidney toxicity) have been severe and in fact stimulated research towards derivative compounds. This has resulted in the development of special drug-dosing protocols, making use of rescue agents like sodium dithiocarbamate,[3] and intensive research towards derivative platinum compounds.[2] The second-generation platinum drug carboplatin $[Pt(C_6H_6O_4)(NH_3)_2]$, is also in routine use and has less toxic side effects than cisplatin. Its much lower reactivity allows a higher dose to be administered (up to 2000 mg/dose).

Later developments have shown that spontaneous (acquired) drug resistance may develop in certain tumours. This is one of the main limitations when treating patients. Such resistance is easily detected in tumour cell lines, so that new drugs can now be rapidly screened. As a result a new group of compounds with different amines has evolved during the last decade. These are often considered as the so-called third-generation drugs. They include platinum(IV) derivatives that can be administered orally,[5] *e.g.* *cis,trans,cis*-$[PtCl_2(RCO_2)_2(amine)_2]$ (R = alkyl). Fig. 1 lists a selected number of clinically used or tried platinum amine compounds of the first, second and third generations. Improved sequence selectivity (to avoid mutagenic side effects) and new structures (to overcome the above-mentioned problems of resistance which patients may develop against cisplatin) may result in better treatment of tumours that are not sensitive enough to cisplatin. In summary, new drugs are sought for two major reasons, *i.e.* to reduce toxicity, and to circumvent resistance.

Structure–Activity Relationships and New Derivatives

It has been possible to formulate structure–activity relationships for platinum compounds. So far, in almost all cases reported, the *cis* geometry of two amines (symmetric, asymmetric, chelating or not), and the presence of at least one N–H group on the amine, as well as leaving groups with a weaker *trans* effect than

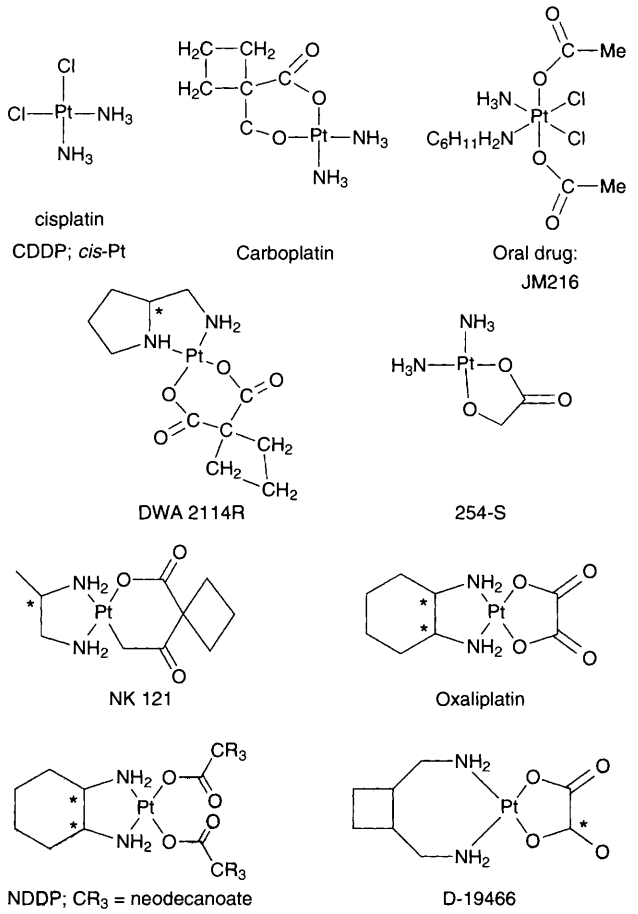


Fig. 1 Schematic structure of some clinically used platinum compounds, as well as derivatives that are at an advanced stage of testing and close to clinical applications

the amine, were found to be necessary.[1,6] Water solubility should be good and toxic side effects should be minimized. Also possible reactions in the blood with ligands containing S-donor atoms should be suppressed.

Most importantly, new compounds should lack cross-resistance to cisplatin and carboplatin. It is known already that this requirement can be reached by using non-ammonia ligands). Therefore, by applying careful variations in: (*a*) the eventually chosen amine ligand at platinum (chelating or not, hydrogen-bond donor, steric effects, possible side arms for secondary DNA interactions); (*b*) the selected leaving groups at platinum (non-toxic; optimal ligand-exchange kinetics; possibility to act as a pro-drug), new drugs can be expected. In addition to these two basic variations some other interesting new approaches in the design of antitumour drugs have appeared during recent years. As a result several new platinum complexes have become available, each possessing one of the following characteristics.

(*i*) They contain (tissue-specific) carrier molecules as ligands for achieving higher drug concentrations, or slower release in or at certain tumour tissues.[7] (*ii*) They are attached to other chemotherapeutic agents, *e.g.* intercalators,[8] or phosphono carboxylates[9] as co-ligands, to obtain some sort of synergistic effect. (*iii*) They contain more than one platinum atom connected by a bridge.[10] (*iv*) They contain radiosensitizers as ligands[11] for use in radiation therapy. (*v*) They have protecting groups that are released (by *e.g.* antibody-linked enzymes) only at the surface of the (specific) tumour cells.[12]

Finally, it should be mentioned that as well as the 'classical' platinum compounds, completely different structural classes of platinum antitumour drugs have also been reported. Some of these compounds possess chemical and biological properties related to those of cisplatin, although the degree of DNA binding (if occurring at all *in vivo*) is not or hardly known. In fact, it might appear that almost any (heavy) metal compound might be potentially antitumour active. Given the many uncertainties, or complete lack of knowledge, in their way of intracellular binding, these compounds will not be discussed in this article. The present feature article is primarily concerned to compounds for which the DNA-binding mode is reasonably known, *i.e.* the classical mononuclear amine compounds and related dinuclear platinum–amine coordination compounds.

Available Biological Ligands and Early Mechanistic Investigations

In theory a large variety of biological molecules could be the target for platinum compounds. Basic coordination chemistry knowledge would predict that S-donor ligands in proteins would rapidly bind and generate the most stable bonds. In addition, binding to lone pairs of nitrogen atoms can be predicted to be strong. Consequently, these types of binding would involve amino acid side chains from cysteine, methionine, histidine, and also the solvent-exposed N^7 atoms of adenine and guanine in double-stranded DNA. In addition to these, in single-stranded DNA and RNA, the N^3 of cytidine and N^1 of adenine would be accessible. These DNA-binding forms are schematically depicted in Fig. 2.

The early mechanistic studies by several groups soon pointed towards a specific cisplatin binding to DNA, eventually shown to be the guanine-N^7 atoms, by a variation of biochemical techniques reviewed previously.[2,3] More than a decade ago we proved that a macrochelate Pt(G-N^7)(G-N^7) can be formed, and even determined its three-dimensional structure in solution.[13] The first quantitative studies, using enzymatic DNA degradation experiments, already made clear[14] that binding at two neighbouring G-N^7 sites in double-standed (ds) DNA occurs very frequently (up to 70% of all Pt on the DNA might be found there), followed by AG sites (up to 20%). Consequently, model studies were undertaken with single-stranded mono-, di-, tri- and larger oligonucleotides containing these nucleotides and sequences. It was soon confirmed that such binding indeed takes place and that it can be studied at the molecular level.

A major next step was the challenge of studying the structure of platinated ds DNA. Den Hartog *et al.*[15] were the first to prove that DNA was kinked at the Pt-binding site. This was later followed by a variety of studies on double-stranded sequences.[3,16–18] Some of these important structural features will be highlighted below.

For all third-generation drugs again binding to a guanine-N^7 site appears to occur. Different compounds, however, appear to have different binding kinetics, and also the structural details of the resulting DNA adducts appear to differ to some extent.[19] This might have important biological consequences, as will be discussed below.

Transport through the Body to the (Tumour) Cell

There is good evidence now that after administration, either by injection/infusion or orally, the drug circulates in the blood, primarily as the chloride (for cisplatin), or as another rather inert form (such as the biscarboxylate in carboplatin). Upon passing through cell walls (either actively or passively[20]) intracellular reactions with peptides and proteins may take place, presumably followed by transfer to nucleic acids.

A very schematic and still somewhat hypothetical picture for this multi-step process is redrawn in Fig. 3. Evidence is becoming stronger that especially the thioether sulfur ligands, from *e.g.* methionine or perhaps oxidized glutathione, play a key role in this transfer of Pt to the nucleic acids.[21,22] Given the strong (kinetic) preference of platinum compounds towards reaction with class B donor atoms (such as those from thiolates and thioethers), binding to nucleic acid bases (a thermodynamic end product) must at least occur partially *via* labile intermediates. These intermediates could be the aqua species, but thioether adducts appear equally if not more likely.[21–23]

Given the strong and almost irreversible binding of platinum amines to DNA bases, most research during the last decade has focused on the structure of such DNA adducts and this will be reviewed below in some detail. More recently,[21–23] attention has been drawn to the kinetics and the competition with other ligands in the cell and the transport through the cell mem-

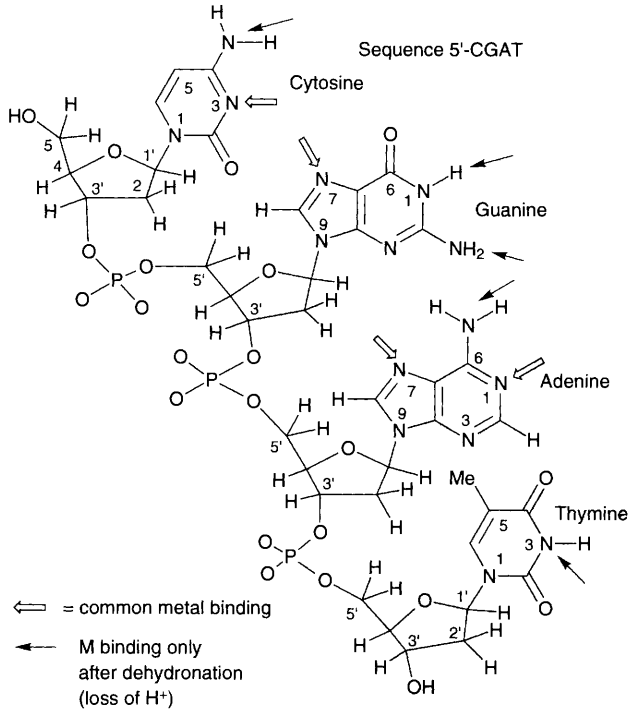


Fig. 2 Possible binding sites for metals (including Pt) on the bases of DNA. In ds DNA in fact only G-N^7 and A-N^7 are accessible from the solvent.

brane.[23] Both inter- and intra-molecular competition studies for platinum–amine compounds with nucleobases and S-donor ligands, such as in *S*-guanosyl-L-homocysteine (see Fig. 4), have shown that only a transfer from a thioether S ligand to a guanine-N^7 does occur. In the case of *S*-guanosyl-L-homocysteine and the nucleopeptides Met-TpG and Met-TpGpG the model compound [Pt(dien)Cl]Cl (dien = diethylenetriamine) first and rapidly binds to the thioether group of methionine; within 2–24 h (at 37 °C), however, migration takes place to a guanine-N^7 site.[23] Similarly, $[Pt(dien)(GSMe)]^{2+}$ slowly reacts with 5′-GMP at 37 °C and pH = 7 to form [Pt(dien)(5′-GMP], as is easily followed by NMR.[23]

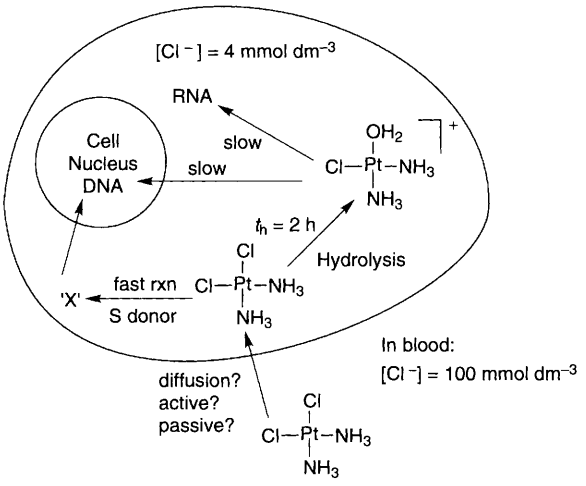
Ligand migrations from a thiolate have not been found as yet, and also ligand transfer to adenine-N^7 has not yet been observed.[23] This observation points towards a delicate balance between thioether-S donors and (only) guanine-N^7 binding, whereas their relative concentrations and that of the platinum species may also determine which products are being formed.

Structural Changes in DNA after Platinum Binding

In an early stage of the mechanistic investigations the possibility of a chelate to two neighbouring guanines was considered, despite the fact that a 17-membered chelate ring had to be part of that structure. After our proof of such a structure by NMR,[13] the structure has also independently been proven by two X-ray structures for the adducts with pGpG[24,25] and CpGpG,[26] respectively. The structure of the CGG aduct, determined in our laboratory, is redrawn in Fig. 5, clearly illustrating the formation of the bis-N^7 chelate and also that the two guanine planes are no longer parallel. In most aspects the compound resembles common square-planar platinum(II) coordination compounds. However, in its intermolecular interactions several unusual interactions, like those found in ds DNA, have been observed. In fact dimers and tetrameric units are kept together by base stacking and by Watson–Crick hydrogen-bonding interactions.[26]

Of course, studies on single-stranded DNA and its platinum adducts cannot yield information about the double-stranded structure; however, major binding features could be revealed for Pt and N^7, allowing studies on a higher level. Therefore, the next stage of the study involved double-stranded DNA, with several attempts to discover whether or not base pairing remains possible after Pt binding. It turned out that monofunctional Pt binding to ds DNA (both on polymeric and oligomeric levels) already induces distortions in the form of bending of the DNA; although the degree of distortion might depend on the actual sequence, the base pairs appear to have remained intact for most sequences studied.[27,28]

For bifunctional binding den Hartog *et al.* were the first to show by two-dimensional NMR analysis[15] that chelation of cisplatin to two neighbouring guanines indeed occurs, that the base pairs remain basically present, and that the DNA bends or kinks by an angle of 40–50°. During recent years this picture has hardly changed and has been confirmed by several other sequences from other laboratories[29,30] and by using other biochemical techniques, such as gel electrophoresis, to study the bending of the DNA.[31] In fact, a weak point in the use of two-dimensional NMR for the determination of such structures is that no direct measurement of the bending angle of the DNA can be obtained. Only the use of XRD in the solid state can provide this information. However, such a structure was searched for during many years without success.



Reactions of cisplatin towards and in the cell

Fig. 3 Schematic picture of cell-wall transport of cisplatin and possible reactions in the cell, leading to binding at DNA in the cellular nucleus

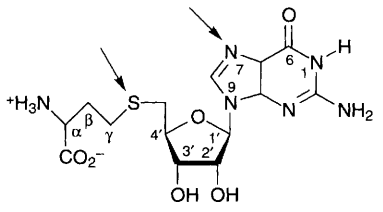


Fig. 4 SGH (*S*-guanosyl-L-homocysteine), an ambidentate ligand for platinum, arrows indicate possible Pt binding; $Pt(dien)^{2+}$ rapidly moves from S to G-N^7 at pH 6.5

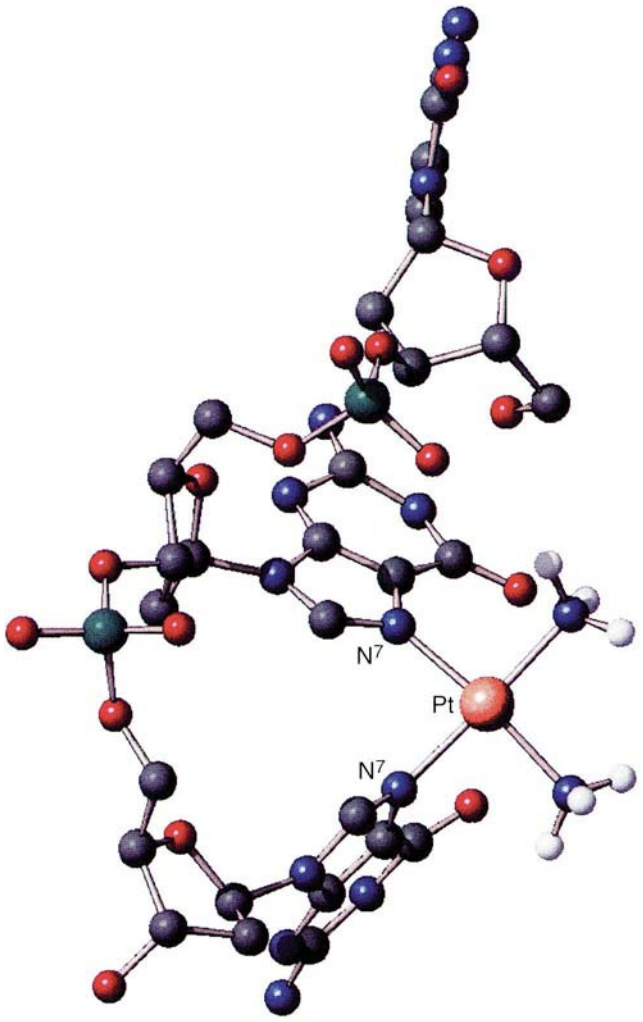


Fig. 5 Projection of the structure of a Pt–GG chelate as found in the X-ray structure of *cis*-$[Pt(NH_3)_2(CGG\text{-}N^7,N^7)]$

Until very recently, no X-ray structure of a ds platinated DNA adduct had been reported, despite several attempts in a number of laboratories. The efforts of Takahara and Lippard now have led to the successful structure determination for the double-stranded synthetic dodecamer d(CCTCTG*G*TCTCC)/d(GGAGACCAGAGG)[32] (G* means platinated G at N^7) and can be considered as a major breakthrough in this area of research. The basic structure is kinked as was predicted,[15,17,18,29,30] with a bending of the helix of about 45°. As a matter of fact only small differences with solution NMR structures are observed. In the first place it appears that the angle between the planes of the two chelating guanines is rather small and as a consequence the Pt ion lies about 1 Å out of the guanine plane; secondly, the DNA conformation at the 5′ side from Pt has changed from B-DNA to A-DNA; earlier NMR and XRD studies on smaller fragments have suggested indications for such a change only at the Pt side. Whether the extension towards the 5′ end is only or mainly a consequence of crystal packing remains to be seen. A projection of the DNA structure, based on the X-ray coordinates of Takahara,[32] is redrawn in Fig. 6. The kink in the DNA is clearly visible, but the stacking and base pairing is only slightly distorted and agrees with that predicted from NMR studies.

Consequences of the Distorted DNA Structure

The next important questions to be addressed deal with the steps *after* the Pt chelation to the neighbouring guanines. Work by Lippard and others has shown that the bent DNA is recognized by DNA-repair systems, such as the Uvr system (in prokaryotes) and the high mobility group (HMG) proteins (in eukaryotes).[33,34] The HMG proteins are known to bend the DNA[35] and it has been suggested that they specifically recognize locally bent and unwound DNA, such as is the case for platinated DNA at neighbouring G-N^7 sites; this may shield the repair.[35,36]

At an early stage it was proven that the progression of DNA polymerase along the DNA chain is blocked at GpG–Pt sites. Also the progression of *Escherichia coli* RNA polymerase was shown to be blocked, indicating that platination has an effect at both replication and transcription.

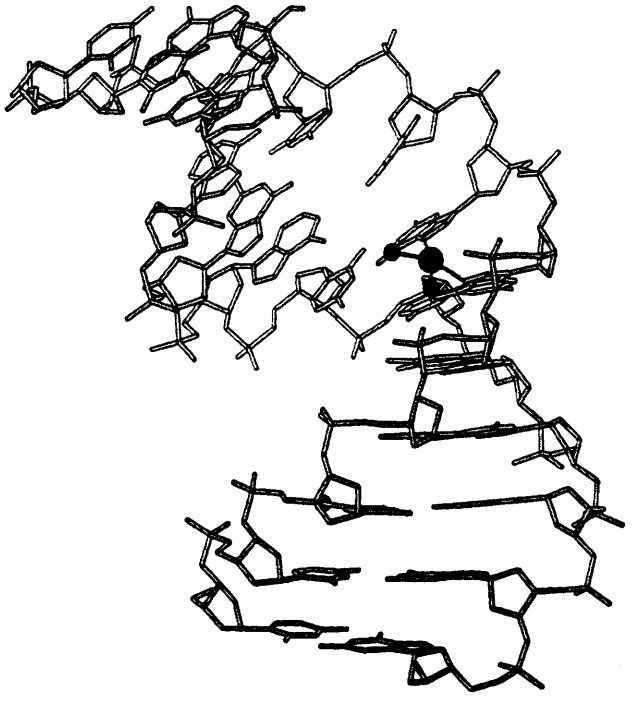


Fig. 6 Projection of the polyanion in platinated ds d(CCTCTG*G*TCTCC)/d(GGAGACCAGAGG) as determined by X-ray diffraction;[32] courtesy of Patricia Takahara (MIT)

Later work has shown that the effect of repair is fundamental for the mechanism. In fact by repairing the damage on DNA caused by cisplatin treatment, the cell might increase the probability of its survival. In experiments with mutants lacking the genes for excision repair or recombination, high sensitivity for cisplatin has been reported.[33,34] Early studies by Brouwer *et al.*[36] have shown that Uvr mutants are very sensitive to platinum treatment. A very well studied system is the UvrABC endonuclease system, capable of DNA damage repair from many origins, including those of cisplatin.[34]

Studies in mammalian cell lines have shown that the d(GpG) adducts are not easily removed by the repair systems, although significant differences can be expected to be present for eukaryotic and prokaryotic excision repair systems.

Consensus is appearing that the 'kinked' DNA is recognized by certain proteins first (the so-called damage recognition, or high-mobility group type proteins), as a result of which the bending of the helix significantly increases; as a consequence the binding of DNA-repair proteins is also prevented or retarded. The family of these high-mobility group proteins is quite large, with molecular masses from 30 to 150 kDa. Work by Lippard and coworkers[37] has shown that a 91 kDa protein isolated from hamster V79 cells only binds to d(GpG) and d(ApG) sites on DNA, and not at the intrastrand d(GpTpG) adducts. In fact, they have demonstrated that one of these HMG proteins has a 100-fold greater affinity for Pt-damaged DNA than for unmodified DNA. This would lead to the suggestion that the simple distorted DNA (like with GG and AG platination), in a way resembles naturally occurring DNA structures (recognized by HMG sequences) and escapes repair reactions. Other mechanisms, such as titrating away essential DNA-binding proteins from their natural sites of action,[39] have been suggested and cannot be excluded.

These and other findings of protein–DNA binding at platinated sites, are likely to have a dramatic effect on the replication, and on the resistance of (certain) tumour cells.[38,40] Spectacular developments can be expected in the next few years, when HMG-type proteins may be co-crystallized with platinated DNA fragments. As a result of such structural investigations, new and improved platinum compounds based on strict geometric requirements are likely to become accessible.

Other Platinum Compounds and Other DNA Adducts

In this feature article so far most attention has been given to mononuclear platinum–amine compounds of a certain structure type. Also we have been focusing on adducts with DNA involving intrastrand GG adducts. It should be mentioned that interstrand adducts have increasingly been studied as well.[41] Such adducts have indeed been found to be formed by cisplatin, albeit in small amounts;[41] similar adducts can also be formed by other, newer compounds, some of which will be mentioned below.

The recently reported dinuclear platinum–amine compounds,[10] closely related to cisplatin, appear to be able to give rise to DNA binding at two different positions, thereby enhancing the antitumour effect. Such compounds might develop into a new generation, and in fact they are expected to be in clinical trials within the next year or two. Some representative structures of such compounds have been redrawn in Fig. 7.

Such dinuclear platinum compounds do react, as expected for platinum compounds with one labile ligand, with DNA and their fragments. Structures with dinuclear platinum compounds coordinated to dinucleotides[42] have been reported, and it has been shown that intrastrand chelation to neighbouring guanines, at least in a single strand, is still possible forming a macrochelate. Most recently, we were able to determine the detailed NMR structure of an adduct of the dinuclear platinum compound 1,1/*t*,*t* (for $n = 4$) with the self-complementary

octanucleotide d(CATGCATG).[43] Much to our surprise no intrastrand adduct was found; instead a spectacular hairpin structure has been found as the only product, which can be described as a 1,2-interstrand adduct. In the adduct the dinuclear platinum–amine unit lies in the minor groove of the dumbell DNA. The structure has been redrawn in Fig. 8. The non-base-paired cytidines are clearly visible at the outer side of the hairpin. Ongoing work here deals with longer and other sequences, and with dinuclear platinum–amine compounds having a longer alkane bridge.

Very recently Farrell *et al.* have shown[10,44] that the reactivity of such compounds with DNA can be influenced by changing some of the ligands (ammonia to pyridine). The steric effects of the pyridines are believed to be responsible for the reduced interaction with calf-thymus DNA. Unwinding of supercoiled plasmid DNA is smaller for the ammonia ligands. Only the 1,1/*t*,*t*/NH_3 compound with $n = 4$ was found to be able to form bis-G-N^7 intrastrand crosslink in supercoiled plasmid DNA.[44] The pyridine derivative cannot induce the B → Z transition in ds DNA, where the two amine compounds given in Fig. 8 can do so.[44]

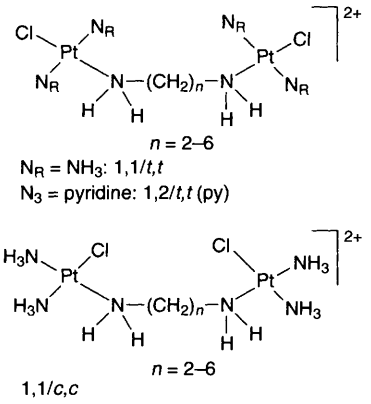


Fig. 7 Schematic structure of some of the dinuclear platinum–amine compounds which show promising activity for cisplatin-resistant cell lines and cisplatin-insensitive cell lines

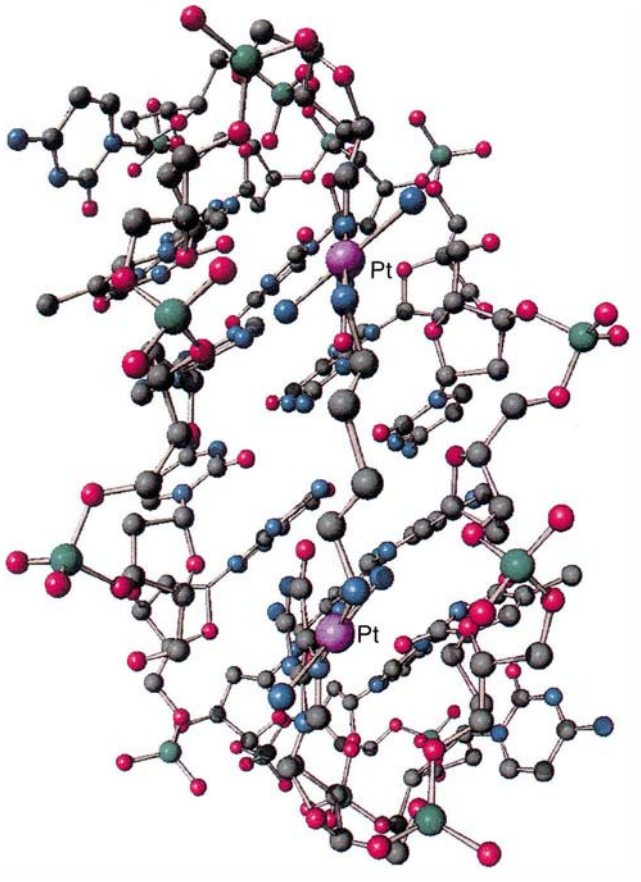


Fig. 8 Structure of a double-hairpin dumbell DNA structure, after chelation of $[-(CH_2)_2NH_2Pt(NH_3)_2Cl]_2$ in the minor groove

As mentioned in the introduction platinum(IV) compounds are also being used as drugs, and the crystal structure of one of them,[45] which is close to clinical application as an oral drug, the so-called JM-216 (see Fig. 1) has recently been reported. A key question is whether or not such compounds are reduced before entering the cell, before binding to the DNA, or not at all. A study by Brabec and coworkers[46] has shown that in the case of binding of *cis*,*cis*,*trans*-$[PtCl_2(NH_3)_2(OH)_2]$ the isolated DNA adducts differ from those formed with cisplatin under the same conditions, and that no external reducing agent is needed for their formation. Whether or not the formed adducts are Pt^{II} or Pt^{IV} is not completely clear, although most evidence points towards slowly formed platinum(IV) adducts.[46]

Outlook

The phenomenon of resistance development upon cisplatin treatment, mentioned in the introduction, is still far from understood, and may have many origins, like decreased Pt uptake by tumour cells, increased levels of S-donor ligands (such as in metallothioneins and glutathione) in the tumour cells, migration of Pt from one site to another on the DNA, increased repair of Pt–DNA damage, or even preferred binding of Pt to other less-critical sequences on the DNA. Also, a decrease in the process of controlled cell killing (apoptosis) could be responsible. Unfortunately, very little information at the molecular level, other than speculations, is available.

Related to the toxic side effects, a completely new area appears to be the chemistry of platinum compounds with rescue agents (usually S-donor ligands) and in particular the reactions of these compounds in combination with other cellular components[47] and their complicated cell-wall transport. Also poorly understood are the deplatination reactions of DNA, or perhaps also migration of Pt units along the DNA chain.[48] In a recent observation we found that under certain conditions (such as the presence of halide ions in solution) the 'classical' bis-G-N^7 adducts (G* means platinated) that cisplatin forms with ds(5′-CCTG*G*TCC)/3′-GGACCAGG) is gradually converted to the interstrand adduct ds(5′-CCTG*GTCC)/3′-GGACCAGG*). Whether this may also happen on biological time scales, or with longer ds DNA sequences, is currently under study.

It is evident that the above topics provide fascinating new possibilities for research in the coming decade. In this respect, the fascinating possibilities of following the reaction of platinum amines and nucleic acids with a strong, new [^{1}H,^{15}N] NMR method, allowing the detection of otherwise invisible intermediates, need definitely to be considered for the future.[49,50] The technique has recently shown to be very useful for studying the competition between L-methionine and 5′-GMP.[51]

In summary, enormous progress has been made in the understanding of the mode of action of cisplatin during the last decade. And there is more to come; it is to be expected that in the next decade improved antitumour drugs will be developed based on the detailed knowledge of the Pt–DNA adducts (and their repair) and on the kinetics of binding to proteins and DNA. In this way, the toxic side effects should be minimized. Detailed knowledge of the reactions of the new platinum compounds on their complicated route from injection or oral administration to the DNA of the tumour cell will be crucial for this development, and will supply a number of major challenges for coordination chemists and many other scientists in the coming years.

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Professor Jan Reedijk (1943) is the Scientific Director of the Leiden Institute of Chemistry, and has been a Professor of Chemistry at Leiden University since 1979. He started his research in the field of platinum coordination chemistry over 15 years ago, while on the lecturing staff of Delft University of Technology. His other research interests include coordination chemistry applications in biomimetics, in homogeneous catalysis and in molecular materials. He is on the editorial board of several scientific journals, including *Chemical Communications*, and has been the Executive secretary of the International Conferences on Coordination Chemistry since 1988.

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