New clues for platinum antitumor chemistry: Kinetically controlled metal binding to DNA

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From the metal ions and metal compounds that are known to bind to DNA, many anticancer Pt(II) and Ru(II) Ru(III) compounds are known to have ligand-exchange kinetics in the same order of magnitude as the division of tumor cells. The present article discusses this process in detail with special attention to cisplatin and related compounds and the cellular binding sites and processes of such compounds. Detailed platinated DNA structures are presented and discussed in light of the mechanistic studies of metal antitumor compounds. It is now known that platinum antitumor drugs eventually end up on the DNA. However, it remains a challenge to understand how (fast) they reach the DNA and how they are removed. The kinetics of ligand exchange around platinum appear to play a crucial role, and the possible role of other ligands as intermediates, especially those with S-donor sites, is of great interest. New types of Pt compounds with additional functionalities influencing DNA binding and kinetics are discussed in the context of steric and H-bonding properties. A comparison is made with more sterically crowded Ru complexes. The effects on activity and correlations with structural and kinetic properties are clues in understanding the biological activities of these classes of compounds.

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eetal ions and metal coordination compounds are known to affect cellular processes in a dramatic way (1). This metal effect influences not only natural processes, such as cell division and gene expression, but also non-natural processes, such as toxicity, carcinogenicity, and antitumor chemistry (2). This article deals with a special aspect of metal biochemistry, namely the reactivity and kinetics of metal coordination complexes in living systems, with a focus on heavy metals like Pt and Ru and their antitumor action (3).

In chemotherapy the key issue is killing the tumor cells, without causing too much harm to healthy cells. Most anticancer drugs act on DNA in one way or another, and the interaction pathways of Pt and Ru compounds with nucleic acids form the core of this article. The successful development of metal-containing anticancer drugs clearly starts with cis-[PtCl2(NH3)2] (see Fig. 1) (4), often referred to as cisplatin. Although the compound was first described in 1845, its anticancer properties were not discovered until 1964, when Rosenberg et al. (5) investigated the effects of electric fields on bacterial growth. By growing Escherichia coli in a solution of NH4Cl and Pt electrodes in sunlight, strong filamentous growth and cell division arrest were observed. They realized that the electric field was not responsible for the cell division arrest, but rather the presence of small amounts of certain platinum compounds, like cis-[Pt(NH3)2Cl2] and cis-[Pt(NH3)2Cl4] caused arrest. Later experiments (6) showed that such compounds form by the slow reaction of the platinum electrodes with the NH4Cl solution in the presence of light and an electrical current (7).

Structure–activity relationships for a class of related compounds confirmed (8) that only those compounds having cis-geometry block cell growth. The most active complex, cisplatin (see Fig. 1), was found to exhibit antitumor activity, whereas its trans isomer showed no such activity. Many derivatives of cisplatin also inhibit growth, and these compounds have at least one N-H group, which is responsible for important hydrogen-bond donor properties (9), either in the approach of the biological target or the final structure (9). A schematic illustration of such hydrogen bonding and its effect on purine bases is given in Fig. 6, which is published as supporting information on the PNAS website, www.pnas.org, whereas an example of hydrogen bonding in a crystal structure (amine to phosphate) (10) is depicted in Fig. 2.

After cisplatin-dependent regression of animal tumors was observed, clinical trials on solid tumors in humans followed rapidly. Phase I clinical trials started in 1971 (11), and Food and Drug Administration approval was obtained in 1978 under the name Platinol. Carboplatin followed with Food and Drug Administration approval in 1989 under the name Paraplatin, whereas most recently oxaliplatin (Eloxatin) also was added for routine treatments of colon cancer (www.cancerbacup.org.uk/info) and others are in phase I and phase II clinical trials. Cisplatin is known to be particularly effective against solid tumor types, such as testicular, ovarian, head, and neck cancers, and against small-cell lung cancer (12) with a cure rate as high as 90%. Typical drug prices are ~$300/g, and sales for carboplatin have been reported to be $480 million in 2001 (www.fda.gov/bbs/topics/NEWS/2002/NEW00825.html).

Most of the well-known platinum anticancer complexes have the general formula (8) cis-[PtX2(NHR2)2], in which R = organic fragment and X = leaving group, such as chloride or (chelating bis)carboxylate. Some representative structures have been included in Fig. 1. Many other active Pt(II) compounds are known now, even with trans geometries, and these will be dealt with below.

The development of cisplatin as a successful antitumor drug is often seen as the prototypical success story. The large number of patients who have been cured after cisplatin treatment of cancer is impressive. However, the fact that the precise mechanism of action remains elusive has resulted in great interest in metal DNA binding generally and cisplatin and its analogs’ binding properties particularly. As a consequence, cisplatin chemistry has provided a fertile ground for exciting (bio)inorganic chemistry research (13).

Quite severe side effects of cisplatin treatment (e.g., nausea, ear damage, vomiting) have stimulated research toward developing less toxic derivatives. These side effects limit the dose that can be administered to patients to 100 mg per day for 5 consecutive days. The nephrotoxicity can be reduced by saline (hydration) and diuresis, and special drug-dosing protocols have been developed, making use of chemoprotecting agents, such as sodium dithiocarbamate (14). The second-generation platinum drug carboplatin, [Pt(C6H6O4)(NH3)2], has fewer toxic side effects than cisplatin and is more easily used in combination therapy. Its low reactivity allows a higher dose to be administered (up to 2,000 mg per day). Carboplatin is used more for ovarian cancer treatment, whereas oxaliplatin is known to be most effective in colon cancer treatment (15). In Japan another second-generation derivative, Nedaplatin (see Fig. 1), is recommended for a variety of cancer treatments, including testicular, ovarian, and cervical cancers (16).

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More recent developments have shown that spontaneous (intrinsic) drug resistance may develop in certain tumors, which is one of the main limitations when treating patients. Such resistance is easily detected in tumor cell lines, so that new drugs can now be rapidly screened. As a result, a new group of compounds with different amines and lacking the classical cis-diamine structure with two leaving groups has evolved during the last decade. These compounds are often considered the so-called third-generation drugs (15, 17–19).

Similar compounds from other group 10 elements (Ni, Pd) do not yield active compounds. The key factor explaining why Pt is most useful clearly relates to ligand-exchange kinetics, which for platinum coordination compounds is the fact that the Pt–ligand bond, which has the thermodynamic strength of a typical coordination bond (say 100 kJ/mol or below), and are much weaker than (covalent) C—C and C—N or C—O single and double bonds (which are between 250 and 500 kJ/mol) (21). However, the ligand-exchange behavior of Pt compounds is quite slow, which gives them a high kinetic stability and results in ligand-exchange reactions of minutes to days, rather than microseconds to seconds for many other coordination compounds. The same holds true for ruthenium coordination compounds, and this kinetic behavior makes such compounds very useful. Although several ruthenium compounds have been reported to show antitumor behavior in cell-line studies (22–25), only one of them has entered clinical trials so far (26). It should be noted that Ru compounds are intrinsically octahedrally coordinated, and even though a cis chelate is possible, the space that axial ligands require prevent it from forming similar structures with DNA as Pt(II) compounds can (Fig. 3). The compounds with three labile ligands, like Ru(terpy), may in theory also bind trans or in a tridentate manner (25).

Another unusual phenomenon deals with the preferred ligands for Pt and Ru ions. Pt(II), and to a lesser extent, Ru(II) do have a strong thermodynamic preference for binding to S-donor ligands. For that reason one would predict that platinum compounds would perhaps never reach DNA, with many cellular platinophiles (S-donor ligands, such as glutathione, methionine) as competing ligands in the cytosol.

Finally, the so-called kinetic trans effect should be mentioned, which is responsible for ligand-exchange reactions on metals ions. The effect is most pronounced for Pt(II) compounds, where it has been studied in great detail (21). The rule can be quite simply formulated as: ligands located trans to another ligand with a strong trans effect (such as...
DNA Is the Target. The activity of cisplatin is closely related to its binding to DNA (35). Highly conclusive evidence for this target was the early observation that cells deficient in DNA repair are hypersensitive to cisplatin (36, 37), suggesting that cisplatin and other drugs bind DNA. Early studies showed that of the four bases the N7 site of guanine is strongly preferred (see also Fig. 6; ref. 20). Many other DNA crosslinking antitumor compounds are known, including cyclophosphamides, nitrogen mustards, nitrosoureas, epoxides, and anthracyclines (38). A drug bound to DNA may interfere with transcription and/or DNA replication mechanisms (39), which may (eventually) trigger processes like apoptosis that lead to cell death (40).

Structures of Pt and Ru compounds coordinated to DNA fragments are numerous and have been reviewed (13). A simple case for ruthenium, showing the hydrogen-bonding importance, is de-
The recognition of the cisplatin GG adduct and subsequent protein binding.
The recognition of the cisplatin GG adduct and 65 and references therein).
double-stranded are known (see refs. 54 double-stranded. Many more structures
resulting in a kink of the DNA when
chelation of fragments is redrawn (64) as a simple
projection in Fig. 4. The chelation of
GG-platinated sites are the
biological consequences of the pro-
ceeds towards creating dinuclear drugs. They
looking for an intrastrand crosslink that
causes only minor distortion of the dou-
ble helix, which reduces the chance of
recognition by repair enzymes. Simple
modeling made clear that a fixed azole
bridge (pyrazole, triazole) would gener-
ate a base-to-base contact of 350 pm.

The X-ray structure of d(CGG) chelated to a cisplatin unit, drawn after Admiraal
et al. (64).

The biological consequences of the pro-
tein binding at GG-platinated sites are the
next challenge to understand (68).

New Types of Drugs
Based on mechanistic findings, coordi-
nation chemists are designing and syn-
thesizing new compounds. A novel
DNA-binding metal compound with an-
titumor activity and clinical efficaci-
ymust fulfill the following key require-
ments: (i) good intrinsic properties, in-
cluding saline solubility and enough sta-
bility to arrive intact at the cellular
target; (ii) efficient transport properties
in blood and through membranes; (iii)
efficient DNA-binding properties but slow
reactivity with proteins; (iv) the
ability to differentiate between cancer-
ous and normal cells; and (v) activity
against tumors that are, or have be-
come, resistant to cisplatin and deriv-
atives. This latter requirement usually
implies a structure that is distinct from
cisplatin-type species.

It is impossible to discuss or describe
all recently studied new compounds, and
only a few will be given below based
mainly on my lab’s work. The first two
groups are based on the classical cispla-
 tin derivatives. The second category
deals with quite different drugs and
DNA binders.

Pt(IV) Compounds and Prodrugs. The so-
called JM-216, a Pt(IV) compound
(Fig. 1; refs. 69 and 70), has been in
routine clinical use as an orally adminis-
tered drug. An important question is
whether such compounds are reduced
before entering the cell, inside the
cell, or perhaps not at all. A study by
Novakova et al. (71) has shown that
in the case of binding of cis,cis,trans-
PtCl2(NH3)2(OH)2 the isolated DNA
adducts differ from those formed with
cisplatin under the same conditions, and
that no external (added) reducing agent
is needed for their formation. Whether
or not the adducts formed contain
Pt(II) or Pt(IV) is not completely clear,
although most evidence points toward
slowly formed Pt(IV) adducts (71).
Work with model bases (72) has made
it clear that reduction can occur, albeit
slowly. Recent work with a variety of
different amines showed that the Pt
redox potential is influenced by the
coordinated amine (73).

Given the toxicity and side effects of
cisplatin, much activity has been gener-
ated on slow-release drugs, such as
through use of a polymer. Such a biode-
gradable polymer can bind cisplatin ana-
logs that are released gradually (74).

A different approach toward prodrugs
has been the formation of membrane-
encapsulated cisplatin, which can be
formed only by repeated freeze-thaw
cycles (75). The resulting products are
highly soluble and appear to result in
slow releases and high activity (76).

Flexible Dinuclear and Oligonuclear Pt Compounds.
From mononuclear Pt
compounds to dinuclear (and oligo-
nuclear) Pt compounds might seem logi-
cal, but it took quite some time before
the dinuclear compounds of the type
given in Fig. 1 were introduced (80,
81). The dinuclear compounds of gen-
eral formula [ClPt(NH3)2(H2N-
(CH2)2)NH2Pt[NH3]2Cl][anion]2 were
found to be active and able to chelate at
two guanines at N7 (82), forming a hair-
pin structure (17) with double-stranded
DNA. Unfortunately, these compounds
were too toxic for clinical trials.

A trinuclear compound (8 in Fig. 1)
was shown to have unique DNA binding
properties (19, 83, 84). The prototype,
also called BBR3464, is active against
melanoma, lung cancer, and pancreatic
cancers and retains active in cisplatin-
resistant cell lines. It is active at 10-fold
lower concentrations than cisplatin and
makes long-range interstrand and intras-
strand crosslinks.

Rigid Dinuclear Pt Compounds. Komeda et
al. (85) followed a different approach
toward creating dinuclear drugs. They
looked for an intrastrand crosslink that
causd only minor distortion of the dou-
ble helix, which reduces the chance of
recognition by repair enzymes. Simple
modeling made clear that a fixed azole
bridge (pyrazole, triazole) would gener-
ate a base-to-base contact of 350 pm.
The prototypical compound is listed as 7
in Fig. 1. When reacted with the model
DNA base 9-ethylguanine (9-Etgua), the
bis adduct (Fig. 10, which is published
as supporting information on the PNAS
web site) beautifully shows the parallel
orientation of the two bases, suggesting that in double-stranded DNA such parallel orientation would be possible.

High activity against a variety of different tumor cell lines was found in the meantime (86), and detailed binding studies of these compounds with DNA fragments were undertaken. In one case an exciting ligand isomerization was found upon DNA binding (87). A reaction with double-stranded oligonucleotides having GG sequences showed that the strand is hardly distorted (refs. 88 and 89; S. Komeda, J. Kozelka, and J.R., unpublished work). A projection of an NMR structure is given in Fig. 5, which illustrates the nonkinked behavior (86, 88).

**Ruthenium and Transamine Pt Compounds.** Although ruthenium antitumor chemistry is more recent than cisplatin and derivatives, the compounds given in Fig. 3 have generated tremendous synthetic activity. My lab has recently modified the azapyridine compounds and made them water soluble (91), which results in compounds with a high antitumor activity. Sadler and colleagues (92) have shown that even organometallic half-sandwich Ru compounds show activity and interesting DNA-binding properties.

Finally, it should be mentioned that many trans-Pt compounds show activity, at least with bulky amines. The interest in transamine Pt compounds is very rapidly growing, and the reader is referred to a review (66). Binding to DNA is prominent, and both interstrand and intrasstrand crosslinking have been reported. An important property for the trans compounds appears to be their activity against tumor cell lines that are not sensitive for cisplatin.

**Kinetics and H-Bonding Properties**

From the previous sections it has become clear that kinetics are crucial to all properties related to antitumor activity and DNA binding. If a potentially active compound with the right geometric and electronic properties does not stay coordinated to the DNA for long enough, no activity will result. Fine-tuning of the compounds with substituents that have steric, electronic, and/or H-bonding implications for DNA binding provides great variety for new compounds.

Recent examples of this principle have appeared in the literature (78, 93), and a very active compound has already resulted from this kinetic approach, as shown in Fig. 1, structure 6 (94). This very simple Pt compound has a structure where the axial attack of incoming ligand at one side of the Pt-ligand plane is significantly slowed down. As a consequence of this geometry, the ligands bind more slowly; indeed, this is a beautiful example of a simple steric control of ligand exchange on simple square planar Pt(II) compounds. This kinetic effect has now also been studied theoretically, including competition between protein and DNA (95).

**A Labeling Application of the Kinetics of Pt-DNA Binding**

The kinetically controlled reactions of Pt compounds to DNA have also resulted in an interesting high-potential labeling application (96). By applying the unit Pt(ethylene diamine) attached to an oligonucleotide, and carrying a fluorescent label, in combination with hybridization to a complementary strand to form double-stranded DNA, it appeared possible to detect specific sequences of DNA in biological samples. Crucial is the fact that the Pt remains coordinated long enough, ensuring that the fluorescent label would only be at the specific DNA sequence. A schematic structure is given in Fig. 11, which is published as supporting information on the PNAS web site.

The moderate stability of the coordination bond between platinum and the guanine-N7, coupled with appropriately slow ligand-exchange kinetics, appears to be a key feature. New applications of this promising methodology have been reported (97).

**Perspective and Future of Metal-Containing Drugs**

Coordination chemistry in living systems is more than just a matter of metal–ligand bond formation and metal–ligand stability. Terminology using the adjective supramolecular, although originally meant for other classes of chemistry also applies here.

Control of metal binding to DNA, by simultaneous coordination and hydrogen bonding has been crucial to my lab’s research and has been a focus of this perspective. Starting with a simple working hypothesis in 1980, we have seen several recent examples to appreciate this approach as useful. The studies have clearly led to the acceptance of the view that:

(i) In metal-DNA binding the kinetics of the metal–ligand binding are more important than the thermodynamic binding.

(ii) The role of additional H-bonding interactions, both in the kinetics of the process, and in the stabilization of the adduct structure, is very important.

It needs no discussion that the above-presented highlights and outlook provide fascinating new possibilities for research in the coming decade. New techniques like specialized MS and NMR including the powerful [1H,15N] method (heteronuclear sequential quantum correlation), which allows following the reaction of Pt amines and nucleic acids and proteins, will allow the detection of otherwise invisible intermediate products (90).

In summary, it is generally appreciated that enormous progress has been made in the understanding of the mode of action of cisplatin, for which kinetics plays such an important role. Application of this knowledge in drug design is close, and it is generally expected that in the next decade improved antitumor drugs will be developed based on the knowledge of the Pt–DNA interactions (and their repair) and on the kinetics of binding of Pt and Ru compounds to proteins and DNA. Kinetic understanding is of great importance for control-
ling the toxic side effects of such compounds. Although questions have been raised about whether the intrinsically weak metal–ligand coordination bond will ever lead to new drug applications (www.callerio.org/forum), the kinetic control of stability is likely to overcome this. The next stage in drug design is likely to be the development of dedicated drugs that comprise the transport (through the membranes), survival in the cell, binding to the DNA, and eventually excetration from the body with minimum side effects. In my perception of this process, both (kinetically controlled) metal coordination and hydrogen bonding will be key factors at the molecular level.

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