Why Does Cisplatin Reach Guanine-N7 with Competing S-Donor Ligands Available in the Cell?

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Received February 16, 1999 (Revised Manuscript Received May 25, 1999)

Contents

Jan Reedijk was born in 1943 in The Netherlands. He is currently Professor of Chemistry at the Leiden Institute of Chemistry, Leiden University, The Netherlands. After obtaining his Ph.D. degree in 1968 (Thesis Methyl cyanide as a ligand) he lectured at Delft University of Technology until November 1979, when he accepted his present position. From September to December of 93 he was a Visiting Professor at Cambridge University. His current research interests include coordination chemistry of transition-metal ions; bioinorganic chemistry (active-site structure and mechanism; models; metal–DNA interactions); applications of coordination chemistry in catalysis, medicine, ion-exchange, and surface chemistry; extended (magnetic, electric) interactions in coordination compounds (dimers, clusters, chains); and molecular recognition and intermolecular interactions (catalysis, biomacromolecules). He has over 700 (co)-authored research publications (1966–1999) and has supervised over 90 postdoctoral fellows and graduate students (1973–1999). Some of his major Professional activities, Honors, and Awards are Director Leiden Institute of Chemistry (1993–present); Member of the International Advistory Board of a number of International Conference Organisations, like the EURASIA Conferences of Chemistry, Macromolecular Metal Complexes Conferences, EUROBIC and ICBIC. He was elected as Executive Secretary of the International Coordination Chemistry Conferences (1989) and acted as President of the Royal Netherlands Chemical Society in 1990. He is a Board member of the Chemistry Research Council (1998 onward). He was awarded the Max Planck Research Award in Chemistry in 1992. He is an Elected Member of the Finnish Academy of Sciences (1997) and also an Elected Member of the Royal Netherlands Academy of Sciences (1996). He is and has been a member of the Editorial boards of over 15 major chemistry journals, such as *Chemical Communications*, *Inorganic Chemistry*, *Journal of the Chemical Society*, *Dalton Transactions*, and most recently including *New Journal of Chemistry* and the *Journal of Biological Inorganic Chemistry*. He is currently one of the Editors-in-Chief of the rapidly growing *European Journal of Inorganic Chemistry* (merger from several European Chemical Society Journals).

I. Introduction

It is beyond doubt that the story of cisplatin, in its development as a very successful antitumor drug, has been a success story. The huge number of patients that have been completely cured after cisplatin treatment of cancer[1] speaks as such. Given the fact that the precise mechanism remains elusive, cisplatin and its several analogues have provided a fertile ground for exciting (bio)chemistry. This paper will deal with a special aspect of platinum chemistry, namely, its reactivity with a group of ligands that is not present in the drug but nevertheless must play a key role in the process of drug distribution in the body, in the mechanism of metabolism of the Pt-antitumor compounds, in the therapeutic effect, and in the serious toxic side effects of cisplatin

A key question, intriguing for many chemists, has been why Pt antitumor compounds do not end up bound at S-donor ligands. An overview of Pt−S interactions relevant for the mechanism of action of cisplatin and related Pt-antitumor drugs is presented. Although it is nowadays generally accepted that DNA platination is the ultimate event in the mechanism of action of platinum anticancer drugs, the precise mechanism has remained elusive. Despite these uncertainties, the fact that the major adduct formed after attack of cisplatin on the DNA is the intrastrand cross-link between N7 atoms of two adjacent guanine (G) residues is undisputed.

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10.1021/cr980422f CCC: $35.00
Published on Web 08/21/1999

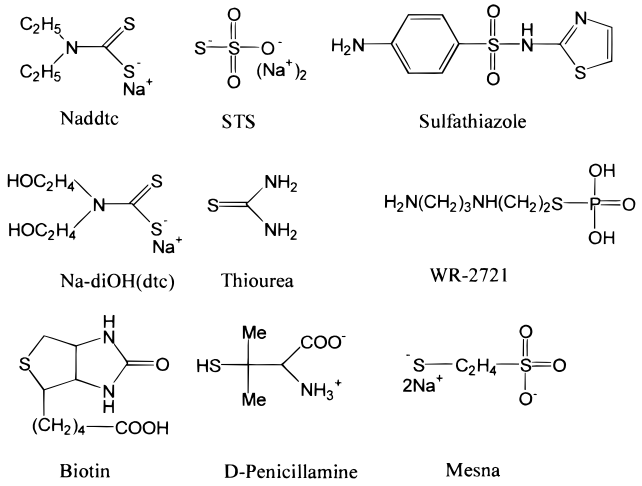


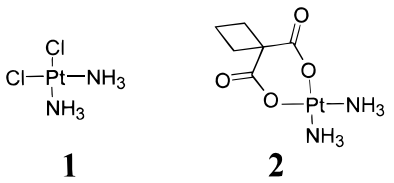
Figure 1. Structures of selected potential rescue and protecting agents.

However, basic coordination chemistry knowledge would not predict this behavior. On its route to the ultimate destination, platinum complexes do also interact with many other biomolecules, especially those containing methionine and cysteine residues.

In fact already in the blood, where the Pt drug is administered by injection or infusion, several S-donor ligands are available for kinetic and thermodynamic competition. Consequently, the so-called "rescue agents" or "protecting agents" have been developed to overcome or reduce binding to such groups, thereby diminishing the toxic side effects. Such agents may in fact revert Pt−protein binding and in this way result in a reduction in, e.g., the kidney toxicity. Biomolecules such as methionine and glutathione (reduced GSH; oxidized G−S−S-G) do interact with cisplatin and analogues and so compete with nucleobase binding (see e.g., ref 10).

Recent results have shown that eventually the Pt-binding to guanine-N7, but not to adenine-N7, is thermodynamically favored (see e.g., ref 10). On the basis of this knowledge, a new strategy toward new cisplatin derivatives has been proposed. In fact, both controlled delivery and activation of the drugs toward DNA binding are now coming within reach.

Cisplatin and its closely related analogue carboplatin (see structures **1** and **2**) have two important shortcomings, namely, (1) *Toxicity*, which is thought

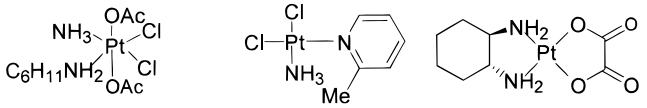


1 **2**

to be related to protein binding of the Pt compounds[2] and might be controlled by administration of protecting agents (so-called rescue agents; usually sulfur-containing ligands) that protect against serious damage. A selection of more or less well-known examples of such protecting (rescue) agents is given in Figure 1. Due to limited understanding in this field, none of these reagents are, as yet, routinely used in patient treatment. However, a quite promising protecting agent for use with cisplatin appears to be WR-2721, which is already registered in a number of European countries.[3] Other recently used agents include mesna (BNP-7787; $[-S-CH_2-CH_2-SO_3]_2$), diethyldithiocarbamate (ddtc), and thiosulfate (sts).[4] Their chemistry will be evaluated in section III of this review. The compound carboplatin (**2**) is less toxic but requires higher doses.

(2) An even more important challenge is to overcome the *development of resistance* of certain tumors to the first- and second-generation drugs.[5] This has resulted in the development of new generations of mononuclear, dinuclear, and even trinuclear platinum compounds, lacking cross-resistance with cisplatin and/or carboplatin. Most recently, a variety of such drugs, some of which are very promising, have become available and urgently require study; they are discussed in another review in this issue.[6]

From the newly applied drugs, in fact the compound JM-216 (*cis*,*trans*,*cis*-$PtCl_2(OAc)_2(NH_3)(C_6H_{11}NH_2)$ (**3**)) is very special because it can be given orally (although patients often have to be hospitalized due to the toxic side effects). In the gastrointestinal tract



3 **4** **5**

this drug is metabolized into several active compounds, probably after being reduced to Pt(II). Whether S-donor ligands have a key role in this reduction process or whether other agents such as ascorbate play a role is likely but not yet determined.

The rather recently introduced[7] compound *cis*-$[PtCl_2(NH_3)(2\text{-picoline})]$ (**4**), initially named JM-473 but more recently renamed as AMD-473 and ZD0473, as described by Giandomenico and Wong,[6] needs to be mentioned as it has clearly been shown that it reduces the reactivity toward ligands, including cellular glutathione.[8]

Although the mechanism of action of cisplatin and its derivatives is in a sense elusive and only partly understood, overwhelming evidence strongly suggests that DNA is the ultimate target in cells,[9,10] where Pt binds primarily (but not only) to two adjacent guanine-N7 sites. Many questions, however, remain as a subject of research in the coming decade. Another review in this issue will deal with the biological events AFTER the DNA binding.[11] For the present review, two separate but related questions will be a central issue: (a) How does the platinum species reach the DNA in the cellular nucleus, (b) How do the several Pt compounds react with protecting agents.

Transport of cisplatin and other Pt species through the cell membranes and possible intermediate binding to proteins both remain largely unknown.[12] In addition, the deplatination reactions of DNA and possible migration of Pt units along the DNA chain[13] are still poorly understood. The process leading to cell killing and the proposed role of apoptosis in these events do require more study.[14] In fact, a simple

question will be addressed, i.e., "*how can platinum reach the DNA*", after administration of the drug, after or despite its reactions with rescue (protecting) agents, after its transport through the cell membrane, and after its possible binding to proteins and peptides as intermediate species.

To date, several of the details of the structure of a relatively small number of cisplatin DNA adducts are known[10,11,13,15−17] and several major new challenges originate from the study of the other in vivo binding processes. The other chemical reactions that occur in vivo, namely, those with proteins and peptides in the blood and in the cell, and those with the protecting agents is at an early stage of understanding and has been studied at the molecular level only during the last 5 years.

In a fundamental approach one has to consider four types of reactive species, all competing for cisplatin, namely, (i) the rescue (or protection) agents, (ii) the cell membrane ligands, (iii) the peptides and proteins, and (iv) the cellular DNA. Fortunately, information on these aspects of in vivo platinum chemistry have become available.[12,18−21] Therefore, the present review will deal with the interaction of (new, active, but also some relevant inactive) platinum compounds (in model fluids; in vitro and in vivo) with cellular components (membrane components, DNA, peptides) and additives (protective agents).

II. Cisplatin as an Antitumor Agent: The Ultimate Target Is DNA

For a proper introduction to the nonexpert reader, a few early accepted key elements in the mechanism for cisplatin are will be summarized here (details are found in refs 9, 10, 22).

After hydrolysis and transport, the binding to DNA is assumed to take place for most platinum compounds of the parent type. Structure−activity relationships for Pt compounds have evolved, and it appears that the cis geometry of amines (symmetric, asymmetric, chelating or not) and the presence of at least one N−H group are necessary.[10,22] Newer platinum complexes have been developed which in a few cases deviate significantly from the classical ones. This development has resulted in the—more general—view that (all) Pt complexes of a certain size and shape, with a certain polarity, capable of (weak, moderate, or strong) binding to biomolecules may show anticancer activity.

For cisplatin and related species, a specific binding at neighboring guanine bases is found in all cases and is especially frequent at guanine-N7 positions; also, the Pt compounds of the newer types seem to demonstrate this preferred binding at G−N7 sites. For cisplatin and a few related species, binding at two neighboring guanines intrastrand chelation takes place, resulting in a specific distortion of DNA, changing its interactions with proteins (see for details the Lippard review[11]).

Some of the newer Pt compounds contain (tissue-specific) carrier molecules as ligands for achieving higher drug concentrations or to have slower release in (or at the surface of) certain tumor tissues. In other cases they are attached to other chemotherapeutic agents, such as intercalators as coligands, to obtain, e.g., a possible synergistic effect.[6] Others contain more than one platinum atom connected by a bridge, and even some *trans*-Pt compounds have been found to be active. For full details the reader is referred to the review of Wong and Giandomenico.[6]

Although in theory many biological molecules could be a target for platinum compounds, basic coordination chemistry knowledge would predict that S-donor ligands present in tissues and, in particular, in proteins would rapidly bind and generate the most stable platinum complexes. Binding to the lone pairs of nitrogen atoms is known to be strong in the absence of S ligands. Consequently, these types of binding would involve amino acid side chains from cysteine, methionine, and histidine. In fact, the solvent-exposed N7 atoms of adenine and guanine in double-stranded DNA would be predicted to be good binding sites for platinum. In addition to these N7 targets, which react with half-lives of a few hours, the N3 of cytidine and N1 of adenine would be accessible in single-stranded DNA.

Although not expected beforehand from chelate-forming principles, a macrochelate Pt(G−N7)(G−N7) with a 17-membered chelate ring can be formed, as verified by NMR and X-ray diffraction sometime ago.[15] More recently, double-stranded DNA structures have been determined also (X-ray, NMR) and the DNA has been found to be distorted and kinked at the Pt-binding site, although the degree of bending may vary depending upon the sequence.[13,17,23,24]

The well-known HSAB theory (now a text-book principle) predicting a very strong (and also rapid) interaction of Pt ions with S-donor ligands in fact would hardly leave any reactivity for the N-donor ligands such as adenine and guanosine, with so many S-donors around in vivo! Nevertheless, and fortunately, the Pt-antitumor drugs finally end up at N7 atoms of guanine. Why and how this process may happen under in vivo conditions will be addressed below.

Upon administration (injection or infusion), the drug circulates in the blood, primarily as the chloride (for cisplatin, **1**), or as another rather inert form (such as the biscarboxylate in carboplatin (**2**)), or the oxalate in oxaliplatin (**5**). Upon circulation in the blood, reactions with proteins and other (rescue, protecting) agents can also take place. Upon passing through cell walls (which is either an active or a passive process[12]), intracellular reactions with peptides and proteins, present in large amounts, may take place. In a final step, presumably, the transfer to the nucleic acids will take place. Given the above-mentioned strong (kinetic) preference of Pt compounds to react with class-B donor atoms (such as those from thiolates and thioethers), binding to nucleic acid bases (apparently a thermodynamic end product) must at least occur partially via labile intermediates.

In recent years, competition studies for Pt−amine compounds with S-donor ligands and nucleobases, such as in *S*-guanosyl-L-homocysteine, and carried out in our laboratory[18−21] as well as others have shown that easy transfer from a thioether S ligand

occurs only to a guanine-N7 site and not from thiolates or to A−N7 sites. So, for *S*-guanosyl-L-homocysteine and for the nucleopeptides Met−TpG and Met−TpGpG, migration takes place to a guanine-N7 site. This observation, as will be detailed below, has indeed provided the first clear evidence that S-donor ligands, including those of protecting agents, may be involved as reaction intermediates.[18−21]

III. Reactions of Platinum Complexes with Protecting Agents

A. Introduction

In section I of this review the concentration-dependent toxicity was mentioned to be an important shortcoming of cisplatin. In fact, this phenomenon had—some 20 year ago—almost been a reason for terminating its clinical application. In fact, toxicity has been associated with competitive protein binding of platinum compounds, and side effects of high-dose cisplatin treatment include nephrotoxicity, ototoxicity, hematological toxicity, neuropathology, and seizures.[25] Nephrotoxicity has perhaps been the major problem in cisplatin treatment, and as a matter of fact, cisplatin could only develop to one of the most widely used anticancer agents after a protocol had been developed to reduce the severe nephrotoxicity, including pre- and post-hydration and mannitol-induced diuresis.[26,27] Nephrotoxicity can also be reduced by application of the cisplatin analogue carboplatin,[28] although even for this compound toxicity remains a problem. In practice, the clinical use of carboplatin is limited by myelosuppression.[28] Therefore, despite much effort to reduce the above-mentioned toxic side effects, a major limitation of the clinical use of platinum complexes[26,27,29,30] remained and several compounds considered as so-called "rescue agents" or "protective agents" have been developed for use in co-administration with the aim to modulate the above side effects of platinum therapy.

Using a known similarity in histopathology of the kidney after Hg(II) and Pt(II) exposure in the rat, Borch and Pleasants[31] did suggest several years ago that a similar mechanism might play a role in the nephrotoxicity of these metals. In these cases, inactivation of enzymes is known to occur by the coordination of Hg(II) and Pt(II) to the thiol residues of proteins. Support for this mechanism is the fact that the total number of protein-bound thiol groups in the kidneys is strongly reduced after *cis*-Pt administration, especially in the mitochondrial fraction.[32,33] Early evidence for such a mechanism was provided by Aull et al.[34] who investigated the inhibition of the enzyme thymidylate synthetase by *trans*- and *cis*-Pt in vitro. This study clearly proved that both platinum isomers do bind to thiol groups of the protein; it was also shown that this interaction could be prevented, but not reversed, by the addition of 2-mercaptoethanol. The enzyme adenosine triphosphatase, which is critical for kidney function, has been proposed as the site of action for nephrotoxicity;[35] however, the high concentrations necessary for this inhibition are unlikely to be reached in vivo.

The known affinity of sulfur for platinum complexes has resulted into investigation of so-called "protecting agents" to ameliorate the side effects of platinum therapy, without reducing its antitumor activity too much. Such nucleophilic sulfur compounds include sodium thiosulfate (STS), sodium diethyldithiocarbamate (Naddtc), glutathione (GSH), *S*-2-(3-(aminopropyl)amino)ethylphosphorothioic acid (amifostine, WR-2721, Ethyol), methionine, cysteine, *N*-acetylcysteine, penicillamine, thiourea, biotin, sulfathiazole, sodium 2-mercaptoethanesulfonate (mesna), and its oxidized S−S-bridged dimer (di)-mesna (BNP-7787). The protective effect of these compounds is either due to prevention or reversal of Pt−S adducts in proteins. A few promising compounds (see Figure 1 for several of their structures) will be discussed below in section III.B and III.C.

B. Some Commonly Used Protecting Agents

The well-known inorganic salt sodium thiosulfate (STS) is known to provide protection from nephrotoxicity when administered in a period between 1 h prior to and 0.5 h after cisplatin injection.[36,37] It has been shown that protein-bound cisplatin cannot be released by STS,[38−40] although STS is able to break the Pt−thioether bond in methionine model systems.[41] A likely explanation for the protecting effect of STS is known to be related to its high concentration in the kidney, where it has been proven to react rapidly with cisplatin, consequently inactivating the drug locally.[39] No routine clinical use of this drug is known as yet.

The salt sodium diethyldithiocarbamate (Naddtc) is effective in reducing several kinds of nephrotoxicity, as well as bone marrow toxicity, and when administered 1−4 h after cisplatin, it does not interfere with the antitumor properties of *cis*-Pt.[42,43] This behavior is in agreement with observations that Naddtc could reverse only the selected Pt−DNA cross-links, *cis*-Pt−adenosine 1:1 and 1:2 adducts and the *cis*-Pt-guanosine 1:1 complex, but NOT (Pt−GG) chelates.[43] Naddtc is capable of reversing the Pt−methionine bond but incapable of reversing the Pt−cysteine bond.[41,44] For a long time thiourea[45] and Naddtc have been the only protecting agents that result in protection against nephrotoxicity when administered after cisplatin treatment, at a time when most of the reactive platinum species have already entered the cells or excreted through the urinary tract. It appears that Naddtc acts as a real rescue agent, i.e., not only by prevention of protein inactivation, but also by repairing of cisplatin-induced damage by dissociating Pt−protein adducts. This hypothesis is supported by the findings that the enzymes fumarase,[38] γ-glutamyltransferase,[40,43] and α_2-macroglobulin[46] are inactivated by cisplatin and can be reactivated by Naddtc. One study describes effective protection from kidney damage when Naddtc is administered 12 h before the cisplatin treatment.[47] Unfortunately, and despite promising results from animal models, Naddtc has not been very successful in the clinic, which may be partly due to its toxicity[48] to the central nervous system.

The third agent to be discussed is mesna. It is a reactive and rapidly excreted thiol, it is commonly

administered orally or intravenously as a uroprotective agent in the ifosfamide treatment,[49] and its potential to reduce cisplatin-induced nephrotoxicity has also been studied. Results of early studies have not unambiguously illustrated its use in reducing nephrotoxicity in animal systems; premature inactivation in the blood stream of cisplatin by mesna has been suggested as a possible cause.[50] More recently, research has been devoted to the disulfide (di)mesna (BNP-7787), which is administered as a prodrug for mesna. The BNP-7787 appeared to have a very low toxicity[51] and undergoes an energy-dependent facilitated intracellular transport in the renal and intestinal epithelial cells.[52] One molecule of BNP-7787 is believed to be reduced enzymatically in the epithelial cells to two molecules of mesna by glutathione reductase;[52,53] this then locally inactivates cisplatin without reducing the antitumor activity.[54,55] The application of BNP-7787 as a protective agent is under investigation in a Phase-I clinical trial.[56]

A special case to be mentioned here is glutathione (GSH); it is present in varying concentrations (0.5−10 mM) in cells and has numerous cellular functions, including the detoxification of chemotherapeutic agents; therefore, it may play a role in modulating cisplatin cell sensitivity.[25,57] Recently, GSH was also shown to protect against cisplatin-induced toxicity in animal models. GSH has been administered before cisplatin,[58] but in another study GSH injections were used prior to and after cisplatin.[59] A clinical phase-I study of GSH with cisplatin has shown that toxicity is significantly reduced in the case of ovarian cancer treatment with cisplatin.[60] No significant effects on the antitumor efficiency were observed, but the renal toxicity was significantly reduced.

C. Amifostine

Currently a most promising and also recently most frequently studied protective agent is the phosphorylated aminothiol amifostine, WR-2721, also called Ethyol. Some if its studies will be summarized in more detail below. Amifostine has recently been registered in several European countries[3,61,62] and was originally developed as a radioprotective agent by the U.S. Army. The amifostine was shown to protect normal tissues from the cytotoxic effects of therapeutic radiation, as well as chemotherapy with preservation of the effect on the of the drug on the tumor. Clinical trials (including phase-III trials) have shown significant decreases in renal, hematologic, and neurologic toxicity without effecting the response rates to cisplatin treatment[63] and reduced duration of thrombocytopenia and hospitalization without interfering with the antitumor activity of carboplatin treatment in phase-I and -II clinical trials.[64]

Over the years, several efforts have been undertaken to understand the mechanism by which WR-2721 reduces nephrotoxicity. It is now generally accepted[3,4] that WR-2721 acts as a prodrug that is transformed into the active species WR-1065 after dephosphorylation by the membrane protein alkaline phosphatase (see Figure 2 for some reaction schemes).

Upon hydrolysis, the formed free thiol species WR-1065 (uncharged) is most likely to be the species

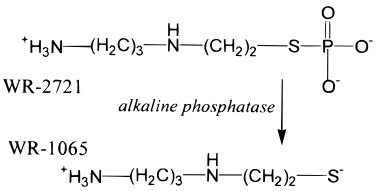


Figure 2. Generally accepted dephosphorylaton of WR-2721.

taken up by the cell and it passes the membrane by passive diffusion.[65] In fact, the selective uptake of WR-1065 in normal cells might be related to known lower levels of alkaline phosphatase of tumor cells.[66] The neutral pH of normal cells compared with the slightly acidic pH of tumors might in some way further favor selective activation.[67] One should realize that WR-1065 can also be oxidized further to form its disulfide or even mixed disulfides of WR-1065 with endogenous thiols of peptides and proteins. Such disulfides have been suggested to serve as an exchangeable pool of WR-1065.[3] Experiments using model systems have indicated that WR-1065 substitutes the Pt−methionine bond much slower than the more nucleophilic modulating agents STS and Naddtc;[68] however, it is a very potent inhibitor of DNA platination.[69] This would suggest that WR-1065 protects through direct interaction with cisplatin and in this way prevents toxicity. Pharmacokinetics of WR-2721 and its metabolites have been investigated in order to optimize the administration protocol. WR-2721 is rapidly cleared from the blood (half-life of 0.8 h), due to the fast conversion into WR-1065. The WR-1065 is cleared from the plasma with a half-life of a few hours, which has been explained by the fast uptake in tissue and to the formation of disulfides that can be detected for a period of over 24 h.[3]

As WR-2721 is generally also well tolerated by patients,[70] it is a promising modulating agent to ameliorate the side effects of platinum therapy; it not only allows a better patient tolerance of current regimes, but also potentially allows improved antitumor efficacy through possible dose escalation. Thus, the prodrug WR-2721 might be quite close to application, and a standard protocol will soon be available. In a recent study, WR-2721 was initially administered 15 min before administration of the platinum complex and dramatically reduced the side effects. Two more subsequent infusions were then given[3] at 2 and 4 h after administration. As yet all studies have been conducted on a limited number of patients, and in particular, randomized clinical trials need to be expanded in order to establish the use of amifostine in worldwide standard cisplatin chemotherapy. Nevertheless, it should be realized that the promising outcome of the above-mentioned clinical studies is hopeful. A recent study has even shown that in mice the antitumor effect of carboplatin is clearly enhanced by use of the co-drug amifostine.[62]

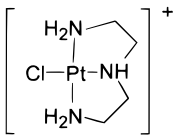
IV. Intermediate Binding Sites for Pt Compounds before Reaching DNA

A. Relevance of Pt–S Binding

In all earlier literature the interactions of platinum species with sulfur-containing biomolecules have

primarily been associated with negative phenomena, such as the development of resistance and the above-discussed toxicity;[71] increasing levels of GSH and/or GSH-transferase might be involved in this process.[71] However, the possible positive effects such as the beneficial effects of Pt−sulfur interaction on the antitumor activity of platinum compounds in preventing side effects and optimal transport will be presented here. The above-mentioned generally known high affinity of platinum compounds for sulfur atoms and the great abundance of sulfur-containing biomolecules in the cytosol and nucleus of the cell have generated the question whether Pt−sulfur interactions perhaps could serve as a drug reservoir for platination at DNA,[22] thus affording an additional pathway toward platination of DNA. In such a process, two reaction paths are possible, i.e., either the spontaneous release of platinum from the sulfur followed by a subsequent reaction with a DNA fragment or the direct nucleophilic displacement of platinum from sulfur by the guanine-N7 group. A few selected model studies used to assess the viability of this idea are briefly discussed below. Competition has been studied intramolecularly in systems where the competing sulfur atom and the nitrogen donor are present in the same molecule. Also, studies toward intermolecular competition will be reviewed. Although Pt−sulfur interactions are kinetically preferred, the binding of Pt with guanine-N7 is often thermodynamically favored, as will be made clear below from both types of studies.

Of course, ideally all such studies should be done with cisplatin; however, simplification is often needed. In fact, a very suitable compound appears to be [Pt(dien)Cl]Cl (dien = 1, 5-diamino-3-azapentane; **6**).



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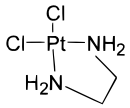

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This model compound is readily available, forms relatively stable complexes with S-donor ligands, has only one substitution step, and is not complicated by (easily occurring) subsequent reactions caused by the trans-labilizing effects of S-donor ligands.

B. Intramolecular Competition Studies

A relatively simple case for the study of possible intermediate binding would be a molecule containing both S- and N-donor atoms, easily and if possible equally accessible. Thus, a relatively easily available ligand *S*-adenosyl-L-homocysteine (SAH, see Figure 3) with two functionalities was selected by Lempers et al.[72] The intramolecular competition between a sulfur-containing amino acid moiety and the nitrogen atom of a nucleobase, however, showed no coordination, neither to the adenine N1 nor to the N7 atom. Instead, a pH-dependent migration of the platinum atom from the sulfur atom to the amine group of the cysteine moiety was observed and vice versa.[72] In a 1:1 reaction at pH < 7, SAH was platinated only at

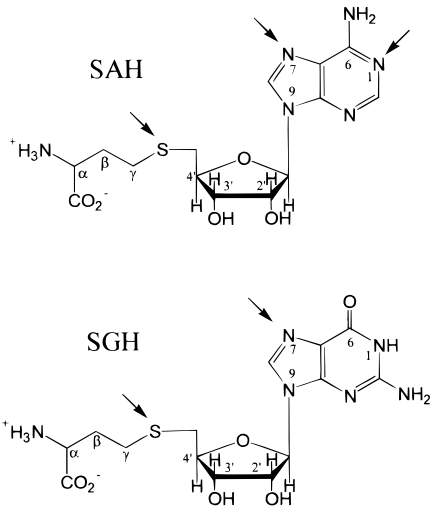
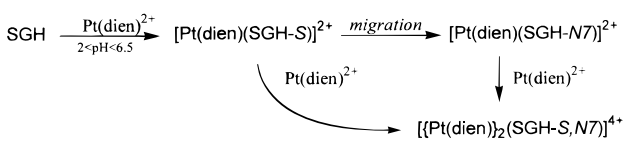


Figure 3. Schematic structures of SAH and SGH.

the sulfur atom to form [Pt(dien)(SAH-*S*)]$^{2+}$ ($t_{1/2}$ = 75 min for 5 mM concentrations). At pH > 7, this product spontaneously isomerizes rapidly ($t_{1/2}$ = 10 min) to [Pt(dien)(SAH-*N*)]$^{+}$ in which platinum is coordinated to the amine of the cysteine group. Furthermore, this pH-dependent migration was shown to be reversible at pH < 5 ($t_{1/2}$ = 2 h). When SAH was reacted with 2 equiv of [Pt(dien)Cl]Cl, the dinuclear complex [{Pt(dien)}$_2$(SAH-*S*,*N*)]$^{3+}$ was formed in which both the sulfur atom and the amine contain Pt.[72] Although lability was shown, migration did not occur.

A much more suitable model system for intramolecular competition appeared to be the molecule *S*-guanosyl-L-homocysteine (SGH, see Figure 3). This molecule had to be synthesized independently, but the outcome appeared to be rewarding.[18] The SGH was reacted with [Pt(dien)Cl]Cl to allow a direct, intramolecular comparison of the reactivity of the sulfur atom with N7 of the more reactive guanine (compared to adenine). Indeed, the species [Pt(dien)(SGH-*S*)]$^{2+}$ does form rapidly upon reaction of SGH with 1 equiv of [Pt(dien)Cl]Cl at 2 < pH < 6.5 ($t_{1/2}$ = 2 h, 319 K), but, as expected, in this case the complex was found to isomerize slowly into [Pt(dien)(SGH-*N7*)]$^{2+}$ with coordination at N7 of guanine ($t_{1/2}$ = 10 h, 310 K). Addition of a second equivalent of [Pt(dien)Cl]Cl yielded [{Pt(dien)}$_2$(SGH-*S*,*N7*)]$^{4+}$. Formation and interconversion of these complexes is schematically depicted in Scheme 1. These reactions do occur in the range 2 < pH < 6.5; at pH values above 7, the dehydronated amino group is also able to coordinate to platinum, resulting in additional complexes with NH_2 coordination. With that study[18] it was it fact shown for the first time that the N7 donor atom can indeed *intramolecularly* replace the sulfur atom in a platinum−sulfur adduct, thereby

Scheme 1. Formation of Several Pt(dien)$^{2+}$ Complexes with SGH at pH Values of 6.5 or Below



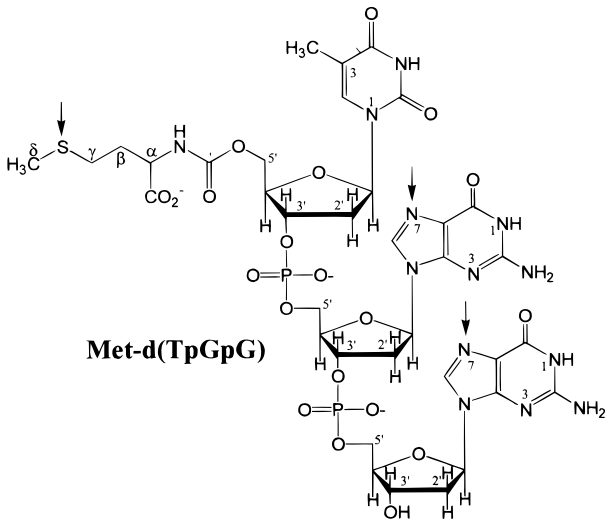


Figure 4. Schematic structure of the nucleopeptide Met-d(TpGpG).

supporting the hypothesis that protein-bound Pt species could potentially act as a drug reservoir.

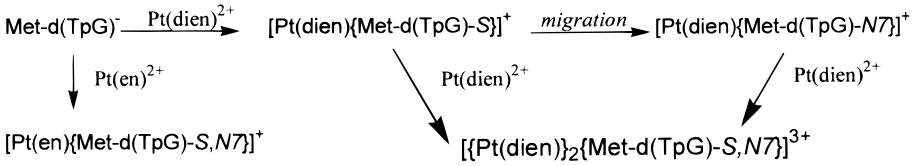
To allow studies at somewhat higher pH, the free amino end group had to be avoided, and therefore, in a large synthtic project two nucleopeptide model species were designed and prepared, i.e., Met−d(TpG)$^-$ and Met−d(TpGpG)$^{2-}$ as the next generation (see Figure 4 for a structure[73]). These two synthetic species contain a methionine linked via its amino function to the 5′ end of a nucleotide moiety. This carbamate linkage prevents coordination at the amino function, making these nucleopeptide models suitable for pH-independent competition studies. As an additional advantage, the thioether sulfur donor now mimics the methionine residues of proteins more closely. Further, the phosphodiester backbone has a closer resemblance to natural DNA than SGH and SAH, giving the model compounds a negative charge and even allowing possible additional hydrogen-bonding interactions with the DNA backbone.

In studies related to the above-described ones, reactions of Met−d(TpG)$^-$ with the platinum complexes [Pt(dien)Cl]Cl, but also with the cisplatin analogue Pt(en)Cl$_2$ (en = ethane-1,2-diamine, **7**), were investigated at pH 7 and 310 K and monitored with both ^{1}H and ^{13}C NMR.[20] The chemical shifts and the relative intensities of the H8 signal of guanine and the SCH$_3$ signals are easily used to deduce information on the course of these reactions. In Scheme 2 the formation of products of the reaction of Met−d(TpG)$^-$ with monofunctional [Pt(dien)Cl]Cl and bifunctional Pt(en)Cl$_2$ is depicted. Also, in this case a rather rapid and initial coordination of Pt to the thioether function is observed, and the formation of the complex [Pt(dien){Met-d(TpG)}-*S*]$^+$ was found to be complete within 2 h. After quite long incubation times, this S-coordinated complex slowly isomerizes, and after 6 days no Pt−S adducts could be detected; in that case, all platinum was found to be coordinated to the N7 of guanine, resulting in the complex [Pt(dien){Met-d(TpG)}-*N7*]$^+$. Repeating this reaction with 2 equiv of [Pt(dien)Cl]Cl yields the product [{Pt(dien)}$_2${Met-d(TpG)}-*N7,S*]$^{3+}$, in which both the thioether and the N7 site of the ligand are platinated. Platination of the sulfur was found to be complete within 2 h, but platination of N7 proceeds much slower and was found to be completed only after 15 h, clearly illustrating the kinetic preference of platinum for the sulfur-donor atom over the N7 of the purine base.

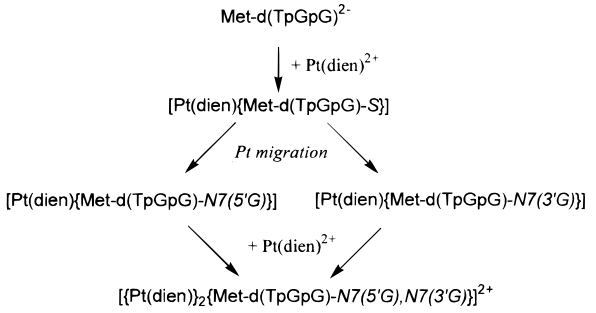
Given the clean experiments with Pt(dien), it was decided to react the nucleopeptide Met-d(TpG)$^-$ with the *bifunctional* compound Pt(en)Cl$_2$. In this case a slightly different reactivity was observed. It was found that coordination of the platinum to the sulfur is slower than for [Pt(dien)Cl]Cl and appeared to be complete within 10 h ($t_{1/2}$ = 100 min). In this case the platination of the N7 of the guanine was detected at the same rate. The nearly simultaneous decrease of signals corresponding to the free Met(SCH$_3$) and G(H8) in the ^{1}H NMR spectrum indicates that the platination of the thioether must be the rate-determining step, followed by a fast chelation step yielding a macrochelate between the sulfur and the N7 of the guanine moiety, [Pt(en){Met-d(TpG)}-*N7,S*]$^+$. The so-formed chelate is stable, and no displacement of the S-bond thioether by N7 was observed, even in the presence of unreacted nucleopeptide.[76] The fact that the reaction with Pt(en)$^{2+}$ results in the formation of a stable *S,N7* chelate, even in the presence of free N7, might seem in contradiction with the Pt(dien)$^{2+}$ results; however, the observation is in agreement with various reports that *S,N7* chelates are quite stable.[74]

In a further extension of this study it was decided to investigate the competition between the sulfur atom and the highly reactive GpG sequence in DNA.[75] For this purpose, the nucleopeptide model Met-d(TpGpG) was used. Thus, reacting dianionic Met-d(TpGpG)$^{2-}$ with [Pt(dien)Cl]Cl[76] initially yields platination at the sulfur atom with a similar rate as that observed for Met-d(TpG), resulting in [Pt(dien)-{Met-d(TpGpG)}-*S*]. In a next step, the platinum coordination was changed toward the N7's of both guanines, resulting in the formation of the monofunctional complexes [Pt(dien){Met-d(TpGpG)}-*N7-(5′G)*] and [Pt(dien){Met-d(TpGpG)-*N7(3′G)*]. Upon addition of an extra equivalent of Pt(dien)$^{2+}$, the final product was found to be the complex [{Pt(dien)}$_2$-{Met-d(TpGpG)-*N7(5′G),N7(3′G)*]$^{2+}$, in which both

Scheme 2. Product Formation and Platinum Migration in the Reactions of Met-d(TpG) with Pt(dien)$^{2+}$ and with Pt(en)$^{2+}$



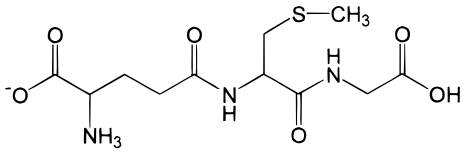
Scheme 3. Product Formation and Ligand Migrations in the Reaction of Met-d(TpGpG) with Pt(dien)$^{2+}$



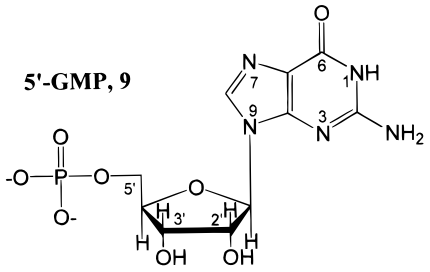
guanines had been platinated (see Scheme 3); however, a small preference for the 3′G was found.[76] Investigations of the reactions between Pt(en)$^{2+}$ and Met-d(TpGpG) are still ongoing.

C. Intermolecular Competition Reactions

Although intramolecular migrations are easy to study under in vitro conditions, intermolecular reactions are much more likely in vivo. All of the above-discussed intramolecular competition studies were performed on models containing both the thioether function and the N7 in the same molecule, thus in relatively close proximity. In the subsequent stage of research, intermolecular competition studies were felt necessary. For this case either methionine or methylated gluthathione (GSMe) (**8**) as the sulfur-containing model was used, whereas guanosine 5′-monophosphate (5′-GMP, **9**) and guanosyl (3′-5′)-guanosine (dGpG, **10**) have been applied as N7-containing models for DNA.

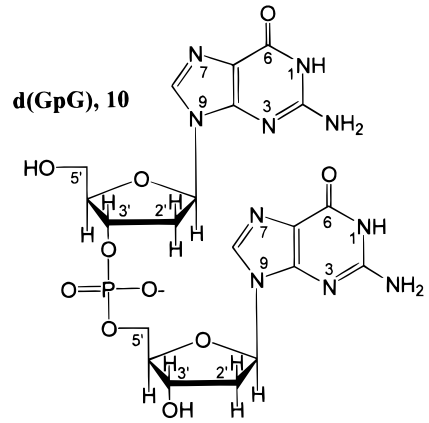


S-methyl glutathione, 8

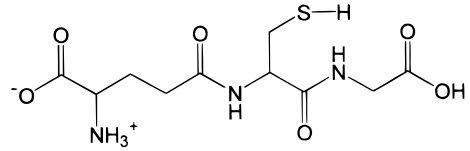


5'-GMP, 9

Research from the Sadler laboratories[77] concentrated on a competition reaction experiment between [Pt(dien)Cl]Cl, *L*-methionine, and 5′-GMP. It was observed that in the first 40 h of the reaction the methionine was platinated to yield [Pt(dien)(Met-*S*)]$^{2+}$, whereas little of the 5′-GMP had reacted. However, in the final stages of the reaction this complex disappears as the platinum binds to 5′-GMP, resulting in the formation of the complex [Pt(dien)-(5′-GMP, *N7*)]$^{2+}$ and free methionine being released. The kinetics of the displacement reaction were studied, and the half-life of the reaction was determined to be 167 h at 310 K. The calculated $H^{\ddagger}$ and $S^{\ddagger}$ values for this displacement reaction are indicative of a substitution mechanism at platinum via an associative mechanism in the so-called five-coordinate transition state. In a related study, the complex [Pt(dien)-(Met-*S*)]$^{2+}$ was also reacted with adenosine 5′-monophosphate (5′-AMP), thymine 5′-monophosphate (5′-TMP), and cytosine 5′-monophosphate (5′-CMP). In this case, no reaction was observed within 12.6 h. Also, the nitrogen of the imidazole was shown not to displace platinum in the reaction between [Pt(dien)-(Met-*S*)]$^{2+}$ and histidine.[77a] However, recently intramolecular migration from a S(thioether) to N(imidazole) has been observed by Sheldrick in the dipeptide histidylmethionine.[77b]



d(GpG), 10



Glutathione, 11

Earlier[72] a similar study was reported about the platination of 5′-GMP by the Pt−S adduct [Pt(dien)-(GSMe-*S*)]$^{2+}$. This study employed the thioether-containing tripeptide GSMe (**8**) and confirmed the intermolecular displacement of the thioether in a Pt−S adduct by 5′-GMP. The kinetics of this reaction were investigated, and the intermolecular rearrangement reaction of Pt(dien)$^{2+}$ to 5′-GMP proceeds slowly at 293 K ($t_{1/2}$ = 179 h). At 308 K, the reaction proceeds faster ($t_{1/2}$ = 31 h). Interestingly, the arrangement was observed only with guanine and not with adenine. Furthermore, the displacement of sulfur in the Pt−S adduct was only observed for the thioether-containing GSMe; when the reaction was performed with glutathione (GSH, **11**), no displacement by the N7 was observed.

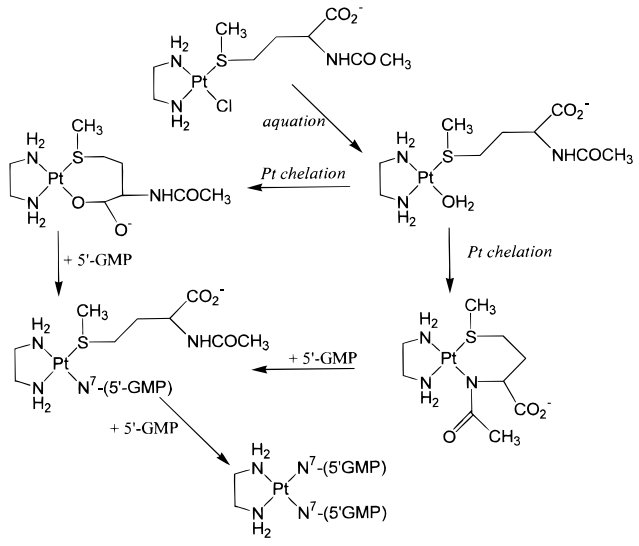
All the above findings confirm the possibility that platinum−sulfur adducts may serve as a drug reservoir; however, the existence of such a reservoir appears to be limited to Pt−thioether type adducts, and transfer to N7 might be limited to guanines.

Of course, the subtle difference between the several Pt complexes on one hand and the several S-donor

ligands on the other hand do not allow general explanations and certainly not predictions. Clearly, more intermolecular competition studies are needed. It appears that reactions of the bifunctional $Pt(en)^{2+}$ are a good basis for further studies.[19] Sadler et al.[19] used the complex [Pt(en)(MeCO−Met-*S*)Cl]NO_3 and reacted it with 5′-GMP and dGpG. In the initial stages of these reactions, an intermediate chelate complex [Pt(en){MeCO−Met-*S*,*N*)] is observed, which reacts via a ring-opening reaction with 5′-GMP and dGpG to form the monofunctional mixed-ligand complexes [Pt(en){MeCO-Met-*S*}{5′-GMP-*N7*}]$^+$ and [Pt-(en){MeCO-Met-*S*}{dGpG-*N7*}]$^+$, respectively; in these cases the chloride is replaced by the N7 site of the guanine.

The so-formed monofunctional adducts appear to be very stable, but very slow substitution of the (MeCO-Met) is observed when the monofunctional 5′-GMP adduct [Pt(en){MeCO-Met-*S*}{5′-GMP-*N7*}]$^+$ is left to react with another molecule of 5′-GMP to form the bis complex [Pt(en)(5′-GMP-*N7*)$_2$]$^{2+}$. Displacement of ([Pt(en){MeCO-Met-*S*}) was also found to be slow for the monofunctional adducts of dGpG, [Pt-(en){MeCO-Met-*S*}{dGpG-*N7*}]$^+$, in which either the 3′G or the 5′G was platinated. The stability of these monofunctional adducts indicate that GpG chelate formation is not a driving force for this displacement reaction.[19] The bis complex [Pt(en)(MeCO-Met-*S*)$_2$]-[NO_3]$_2$ was also prepared, and the S-bound MeCO is more rapidly displaced from this complex by either 5′-GMP or dGpG, giving rise to the formation of the stable related monofunctional adduct. The relevant reactions of the complexes [Pt(en)(MeCO-Met-*S*)Cl]-NO_3 and [Pt(en)(MeCO-Met-*S*)$_2$][NO_3]$_2$ with 5′-GMP are schematically given in Scheme 4.

Scheme 4. Reaction Scheme for [Pt(en)(MeCO-Met-S)Cl]NO_3 and [Pt(en)(MeCO-Met-S)$_2$][NO_3]$_2$ with 5′-GMP (Adapted from Ref 19)



As will be clear from these data, our knowledge of the interaction between platinum complexes and sulfur-containing peptides and the competition between S- and N-donor atoms remains limited and much more information is likely to develop from studies in related fields. To be mentioned here is at least the work of Siebert and Sheldrick.[78] They investigated the pH-dependent competition between *N*,*S* and *N*,*N*′ chelation in the reaction of [Pt(en)-(H_2O)$_2$]$^{2+}$ with methionine containing di- and tripeptides. It was made clear that in met−Hgly and met−gly−Hgly, peptides in which the methionine is located at the aminoterminus, the κ^2N(amino),*S*(thioether) chelation mode dominates at pH < 8.6 whereas the κ^2N(amino),*N*′(amide) dominates at higher pH. For the peptides gly−Hmet and gly−met−Hgly the κ^2N′(amide),*S*(thioether) chelate is observed while at high pH (>7.4) the κ^2N(amino),*N*′(amide) chelate dominates for these peptides as for the peptides with the N-terminal methionine. For the tripeptide gly−gly−Hmet, only one major product is observed: the κ^2N″(amide),*S*(thioether) chelate. That product is stable at pH < 10.6, and no κ^2N(amino),*N*′(amide) chelate was observed. This study[78] illustrates the importance of *N*,*S* chelates and their sequence- and pH-dependent conversion to *N*,*N*′ chelates for the reaction of platinum complexes with methionine-containing biomolecules. It appears that studies on this type of peptides provide important kinetic evidence for such migrations of platinum species.

D. Future and Relevance of Studies in Competition Reactions

The exciting question, not yet answered, deals with why thiols react so different compared with thioethers, at least with respect to platinum. Evidently the neutral thioether has a very high affinity for the square planar Pt(II) ion, although its thermodynamic stability might not be very high, as the bond can be reverted and changed to guanine-N7, which is formed more slowly. Nevertheless, the Pt−N7(guanine) bond appears to have a higher thermodynamic stability (in comparison to adenine), and this may be caused by the additional H-bonding interactions; such H bonds are absent in corresponding adenine-N7 species.[10,22]

Thiols present in vivo evidently do not reduce or stop the antitumor activity, but the origin is not yet understood. Also in this case a key role for the thioether binding may be in operation when it is assumed that the rapid thioether binding protects the Pt species from the attack by thiols or at least slows down the reactions of it.

Although it is known that Pt−methionine species are not antitumor active, methionine does play an important role in the metabolism of cisplatin. The bischelate [Pt(Met-*S*,*N*)$_2$] has been isolated from the urine of patients treated with cisplatin several years ago,[79] but intermolecular competition studies have unambiguously shown that formation of a bifunctional G−N7,G−N7 adduct for the cisplatin analogue $Pt(en)Cl_2$[19,80] can occur.

It appears that now competion studies between thiols/thioethers and intact double-helical DNA are required to find out whether the ultimate formation of the Pt−GG chelate is a driving force that can overcome or control the Pt−S interactions. But in such studies attention should also be given to physiological conditions, as other metals in the cell are likely to play a (co)role in the disruption of Pt−S

bonds. Addition of transition metals such as Zn^{2+} or Cu^{2+} can cleave even the Pt−S bond in thiolated terpyridine−platinum complexes at neutral pH,[81] as was recently reported.

V. Summary, Final Remarks, and Outlook

The previous sections of the review have shown a subtle balance between Pt−S and Pt−N binding in biological systems. This balance clearly is the answer to the question raised in the title of this review. However, there is much more to be learned from the data presently available. A better understanding of this kinetic and thermodynamic balance will soon allow the possibility of application in administration of Pt drugs and at a later stage even in drug design based on this knowledge. As discussed above, a critical process is the delivery of the Pt species at the DNA, where it must stay long enough to play its biological roles of preventing cell division and surviving DNA repair. After injection/infusion (or oral absorbance) of the drug on route to the DNA, the Pt species has to survive many attacks of S-donor ligands. In certain cases, as shown in section III, such an S-donor ligand can also be beneficial in preventing certain toxic side effects. Control of the reactivity of S-donor ligands is crucial, and this may be applied in the design of new types of drugs.

Some potential new options are presented now. First of all, one can consider new drugs that do not contain S-donor ligands. Such compounds do react, in vivo, slowly (i.e., much slower than cisplatin) with competing S-donor ligands. Compounds of this type might be, e.g., the earlier mentioned compound *cis*-$[PtCl_2(NH_3)(2\text{-picoline})]$, AMD-473.[8,82,83] In another process, targeting of the drugs, e.g., with intercalators to improve binding to DNA, might result in less loss by S-donor binding.[84] In a different approach, one could design new drugs that do contain S-donor ligands of optimal kinetic and thermodynamic stability, as discussed above.[85−87] Finally, one could think of totally new Pt(IV) compounds that need to be activated via reduction but now using cellular thiol compounds.[7,88] Also, the new species *trans*-$PtCl_2(NH_3)L$ ($L = NH_3$,quinoline) reported by Farrell where a guanine to methionine reaction has been considered for nonclassical Pt species is mentioned here.[89]

From what is known so far, steric effects as well as electronic factors should play a role in mastering the reactivity of square planar platinum complexes. The presence of bulky, planar amine ligands in *cis*- or *trans*-$[Pt(anion)_2]$ complexes and their orientation with respect to the coordination plane, as well as their substituents, can reduce the rates of DNA binding or thio binding compared to aliphatic ammine and amine complexes. Substituents close to the amine or imine coordination site should be expected to reduce axial substitution reactions at Pt.[8] Given the fact that there is little doubt that DNA platination is the most important event in the mechanism of action of platinum anticancer drugs, research devoted to the process of (rapid) formation of the major adduct (GG) as an intrastrand cross-link between N7 atoms of two adjacent guanine (G) residues is likely to gain importance.

Several studies over the past decades have made it clear that the undesired side effects of platinum compounds do have an exciting molecular basis. This research has stimulated new activities dealing with Pt compounds and S-donor protecting agents and especially the study of the reactions of these compounds in combination with other cellular components and their rather complicated cell-membrane transport. In fact, the results summarized and discussed in this review should have made it clear that even though we have learned a lot on new reactions in which S-donor ligands are involved, many challenges do remain for future research and many questions are as yet unanswered. To serve readers and future generations of researchers, some of the important and newly raised questions are given here. (1) Could other physiologically relevant metal ions, such as Zn and Mn, interfere with the processes of platination and deplatination of proteins and nucleic acids? (2) Could protecting agents have direct chemical interactions with platinum compounds (such as the drugs cisplatin and carboplatin; transplatin)? (3) Which interaction products of protecting agents and cisplatin are formed in vivo (structure, kinetics)? (4) Assuming that interactions between cisplatin and rescue agents do indeed occur, can these be influenced by reaction conditions: pH, time, isomer (cis/trans), co-ligands, other ligands, buffer influence? (5) To what degree do Pt−rescue-agent interactions (and the resulting products) interfere with the binding of the Pt compounds in cells (especially with nucleic acids and/or proteins)? (6) To what degree, if at all, could protecting agents and sulfur-containing peptides cause in vivo deplatination of DNA and proteins? Do such interaction products still have an antitumor activity on their own, and may this knowledge be used to the development of new drugs?

There is little doubt that detailed answers to such questions will require a highly original and innovative approach and also input, not only from chemists, but also from the medicinal, biological, and toxicological field.

VI. Acknowledgments

The author is indebted to the EU for a grant as Host Institute in the EU Program Human Capital and Mobility (1994−1997). Also support and sponsorship concerted by COST Action D8/00097/97 (Chemistry of Metals In Medicine) is kindly acknowledged. The author wishes to thank Johnson & Matthey (Reading, U.K.) for their generous loan of K_2PtCl_4. This work has been performed under the auspices of the joint BIOMAC Research Graduate School of Leiden University and Delft University of Technology. Finally, this research has been financially supported by the Council for Chemical Sciences of The Netherlands Organization for Scientific Research (CW-NWO).

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CR980422F