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INTERACTIONS OF PLATINUM AMINE COMPOUNDS WITH SULFUR-CONTAINING BIOMOLECULES AND DNA FRAGMENTS

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I. History of *cis*-Pt as an Antitumor Drug

Over the past 20 years, *cis*-diamminedichloroplatinum(II), known for more than 145 years, has emerged as the classic compound in the context of antitumor drug therapy. Because it is generally accepted that binding of the compound to DNA is a major requirement for its biological activity, scientists have focused their attention especially on platinum–DNA interactions. In the present review, the latest results

concerning these interactions are briefly described. However, most attention will be given to the reactions of this and related platinum compounds with S-containing biomolecules. Although not directly relevant in antitumor activity, such interactions are considered to have an overall inhibitory effect on the drug action, and are therefore of considerable importance.

The compound [*cis*-$PtCl_2(NH_3)_2$], here referred to as cisplatin, or *cis*-Pt,[1] and its trans isomer were known in the last century. Renewed interest in these compounds was evoked by the results of Rosenberg. As a physicist, he was impressed by the similarity between the mitotic spindle figures and the field lines of a dipole. This led him to investigate—in 1962—how electric fields may interfere with cell division of cultured bacterial cells. The field generated between platinum electrodes seemed to stop cell division without hampering cell growth, in this way inducing filamentous growth (*1*). Subsequent experiments by the same research group soon made clear that this curious phenomenon was caused by small amounts of compounds such as [*cis*-$PtCl_4(NH_3)_2$], formed during electrolysis by interaction of the electrolyte NH_4Cl and the "inert" Pt electrodes (*2*). After this important discovery, numerous Pt (II) and Pt (IV) compounds were found to show similar effects on bacterial growth (*3*). Surprisingly, only the cis and the trans isomer appeared to be effective. Subsequently, the antitumor activity of these and other platinum compounds have been studied. In particular, regarding the effect on tumors induced in animals, such as sarcoma, 180 and leukemia L1210 in mice (*4, 5*) [*cis*-$PtCl2_2(NH_3)_2$] (Fig. 1) turned out to be a very active compound (*6*). In many cases a total regression of the tumors was observed. Clinical trials of *cis*-Pt started in 1971 (*7*). Currently, *cis*-Pt is routinely used and has been particularly successful in the treatment of testicular and ovarian cancers (*8, 9*). The severe renal toxicity exhibited by the compound can be largely circumvented by the use of diuretics and prehydration. In 1978, *cis*-Pt was officially approved as a drug in the United States, and by 1983 this first metal antitumor compound had become the biggest selling antitumor drug. Moreover, the use of *cis*-Pt led to major improvements in response rates for head, neck, and lung tumors, even though responses to chemotherapy are often of limited duration for these tumors (*9, 10*). *cis*-Pt is also being used in combination therapy with antitumor drugs such as vinblastine (*11*), bleomycin (*11*), and adriamycin (*12*), or in combination with radiotherapy (*13*). For testicular and ovarian cancer the progress in the curing of these tumor types, effected by the use of *cis*-Pt, is

[1] See the complete list of abbreviations at the end of this review.

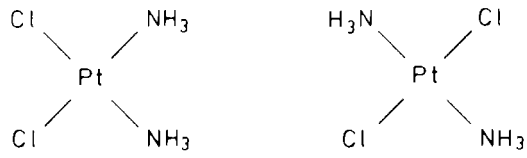


FIG. 1. Structure of *cis*-Pt (left) and *trans*-Pt (right. The trans isomer is not antitumor active.

spectacular (*10*), and especially for early recognized testicular cancer the cure rate is approaching 100%.

Besides *cis*-Pt other platinum compounds have been synthesized and tested for antitumor activity (*14–16*), with the major aim of obtaining higher solubility, better antitumor activity, and lower toxicity. The synthesis of most of these platinum compounds is largely governed by two empirical rules, concerning the trans-labilizing power of the coordinated ligands and the difference in platinum–ligand bond strength. All studies on these derivatives have made clear that there are a number of structural requirements that have to be fulfilled for a complex to show antitumor activity. These are as follows:

1. All complexes should have the cis geometry, with the general formulas [*cis*-$PtX_2(Am)_2$] for Pt(II) and [*cis*-$PtY_2X_2(Am)_2$] for Pt(IV) compounds (*14*). This geometric restriction is automatically answered for didentate amines such as ethylenediamine.

2. The active Pt(IV) compounds are octahedrally coordinated and possess axial bound chloride or—to improve the solubility—hydroxo ligands, i.e., two Y ligands in the trans orientation. These compounds are far more inert than the corresponding Pt(II) compounds that lack these axial ligands. Most likely the Pt(IV) compexes are reduced *in vivo* to the corresponding Pt(II) complexes, which are in fact the active species (*17–19*). They can therefore be considered as a type of "prodrug" that requires *in vivo* activation (substitution and reduction) to the square-planar Pt(II) compounds to exhibit antineoplastic activity. This hypothesis is supported by the observation that platinum(IV) compounds are unable to react with DNA under ambient conditions (*19*), and that appreciable amounts of Pt(II) derivatives can be detected in the urine of Pt(IV)-treated patients(*18*).

3. The ligand X is an anion that should have intermediate binding strength to Pt(II). Complexes with labile anions such as NO_3^- or ClO_4^- are usually highly toxic (*14–20*). Complexes with strongly bound anions are inert, although in some cases the dissociation of these anions appears to be activated *in vivo* (*15, 21*). Examples of effective anions are

Cl^-, SO_4^{2-}, citrate(3−), oxalate(2−), and other carboxylic acid residues.

4. At least one of the two cis amine ligands should have an N—H group (*14*). All compounds lacking this property have been found to be inactive; however, it cannot be excluded that other compounds, acting through a different mechanism, will show antitumor activity. This N—H group is likely required for a hydrogen bond donor function, although steric effects cannot be excluded *a priori*.

A series of very promising "second-generation" platinum antitumor drugs that answer the above-mentioned characteristics is now known and several of these are ready for use in many clinics. A few examples of these compounds, all of which fulfill the above requirements, are shown in Fig. 2 (*22*). There are a few exceptions among antitumor-active Pt compounds that seem to deviate from these empirical rules in having only one anionic ligand. As examples, compounds of the general formulas [Pt(diam)(R′R″SO)Cl]NO_3 (*23*) and [*cis*-Pt$(NH_3)_2$(N-het)Cl]Cl (*24*) can be mentioned. Both classes of compounds are cationic in nature, water soluble, and have promising antitumor activities. Figure 3 contains two examples of these new compounds.

Currently, there are interesting new approaches in the design of antitumor drugs; a variety of platinum complexes are being designed

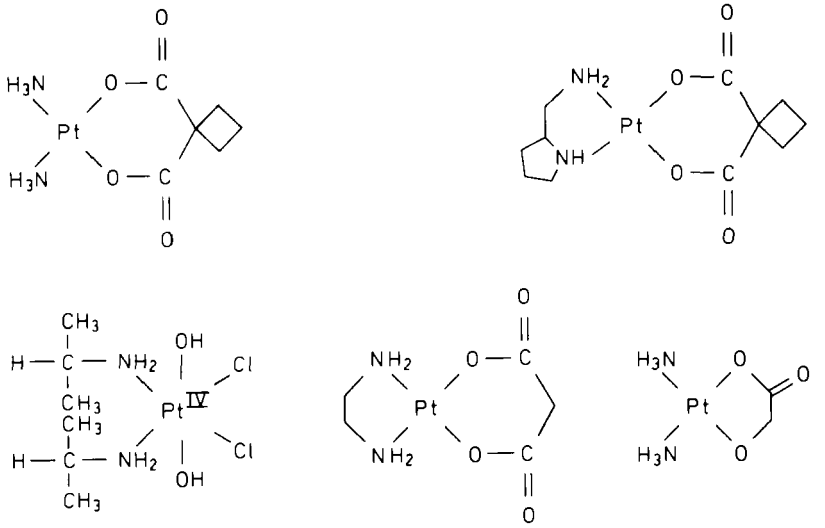


FIG. 2. Structure of some promising platinum antitumor drugs. CBDCA (upper left) is already routinely used in the clinic (*22*).

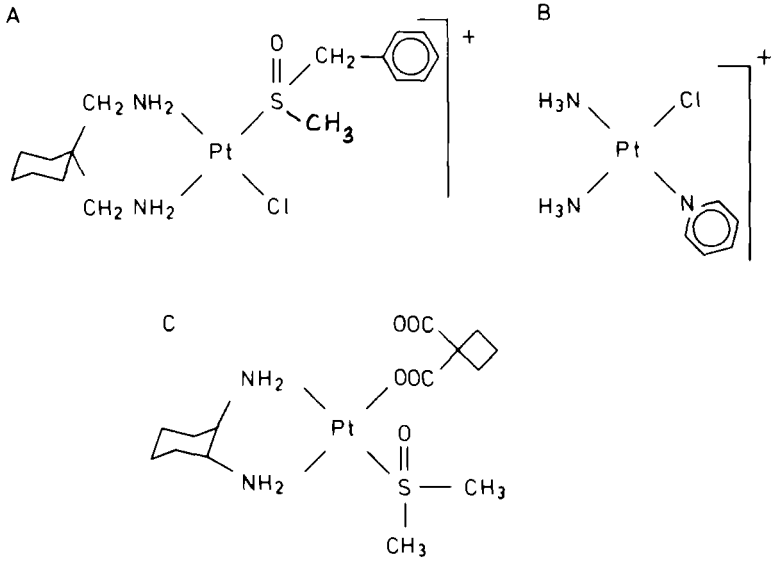


FIG. 3. Structure of [*cis*-Pt(damch)(MeBzSO)Cl]$^+$ (A), [*cis*-Pt$(NH_3)_2$(*N*-pyridine)Cl]$^+$ (B), and [*cis*-Pt(DACH)(Me_2SO)(CBDCA)] (C).

having the following characteristics: (1) containing carrier molecules as ligands for achieving higher drug concentrations, or slower release in tumor tissues (*25*); (2) containing other chemotherapeutic agents, such as intercalators (*26, 27*) and phosphono carboxylates (*28*) as coligands in the hope of obtaining some sort of synergistic effect; (3) containing more than one platinum atom (*29, 30*); and (4) containing radiosensitizers as ligands (*13, 31, 32*) for use in radiation therapy.

II. Aqueous Solution Chemistry of *cis*-Pt

When *cis*-Pt is dissolved in water, the labile chloride ions are slowly replaced by water molecules (hydrolysis) in a stepwise manner as shown in Eqs. (1)–(7) (*33–35*). The whole process of hydrolysis takes a few hours at 37°C.

$$[cis\text{-}Pt(NH_3)_2Cl_2] + H_2O \longrightarrow [cis\text{-}Pt(NH_3)_2Cl(H_2O)]^+ + Cl^- \quad (1)$$

$$[cis\text{-}Pt(NH_3)_2Cl(H_2O)]^+ + H_2O \longrightarrow [cis\text{-}Pt(NH_3)_2(H_2O)_2]^{2+} + Cl^- \quad (2)$$

$$[cis\text{-}Pt(NH_3)_2Cl_2 + OH^- \longrightarrow [cis\text{-}Pt(NH_3)_2Cl(OH)] + Cl^- \quad (3)$$

$$[cis\text{-}Pt(NH_3)_2Cl(OH)] + OH^- \longrightarrow [cis\text{-}Pt(NH_3)_2(OH)_2] + Cl^- \quad (4)$$

$$[cis\text{-}Pt(NH_3)_2Cl(H_2O)]^+ \longrightarrow [cis\text{-}Pt(NH_3)_2Cl(OH)] + H^+ \quad pK_a = 6.85 \quad (5)$$

$$[cis\text{-}Pt(NH_3)_2(H_2O)_2]^{2+} \longrightarrow [cis\text{-}Pt(NH_3)_2(OH)(H_2O)]^+ + H^+ \quad pK_a = 5.93 \quad (6)$$

$$[cis\text{-}Pt(NH_3)_2(OH)(H_2O)]^+ \longrightarrow [cis\text{-}Pt(NH_3)_2(OH)_2] + H^+ \quad pK_a = 7.87 \quad (7)$$

The relative amounts of all these Pt species vary as a function of the pH and the chloride concentration. Only platinum species with a coordinated water molecule are regarded to be reactive, because, in contrast to coordinated chloride or hydroxide, this ligand can be easily substituted by other donor molecules. Hydroxo species are formed as indicated in Eqs. (3)–(7) (*34, 35*), with [*cis*-$Pt(NH_3)_2(OH)_2$] as the stable end product in basic solution (*36*). It should be noted, however, that this species very easily dimerizes and trimerizes at higher concentrations, producing ions such as [*cis*-$Pt(NH_3)_2]_2(\mu\text{-}OH)_2$ and [*cis*-$Pt(NH_3)_2]_3(\mu\text{-}OH)_3$, as has been proved with, e.g., ^{195}Pt NMR spectroscopy (*36a, b*). Very recently, accurate pK_a values have been presented for the (de)hydronation equilibria (*36b*); the pK_a values have been added to Eqs. (5)–(7). Miller and House (*36c*) have accurately determined the kinetic parameters for the several hydrolysis reactions. They concluded that acid hydrolysis of *cis*-Pt *in vivo* is unlikely to proceed beyond [*cis*-$Pt(NH_3)_2Cl(H_2O)]^+$.

In blood plasma, the chloride ion concentration is sufficiently large (about 100 m*M*) to prevent *cis*-Pt hydrolysis, and the neutral platinum species most likely crosses the cell membrane. Inside the cell the chloride ion concentration is much lower (about 4m*M*), which allows for hydrolysis (*35, 37*). Because water is a far better leaving group than chloride or hydroxide (*38, 39*), the aqua species are most likely the reactive form of *cis*-Pt *in vivo*. Thus hydrolysis is the rate-limiting step in the reaction of *cis*-Pt with biomolecules such as proteins, RNA, and DNA (*40*).

In fact, the very recent ^{195}Pt NMR results of Bancroft *et al.* (*41*) indicate that, in agreement with Miller and House (*36c*), most likely [*cis*-$Pt(NH_3)_2Cl(H_2O)]^+$ is the predominant species that reacts with biomolecules (at least with DNA). Other Pt amine compounds that are antitumor active have different kinetics of the hydrolysis reactions, and usually react much slower. The second-generation drug CBDCA (Fig. 2) is known to hydrolyze (in a 1 m*M* solution) with a half-life at 37°C of a few days (*41a*) (compared to only 1 hour for *cis*-Pt).

III. Antitumor Activity and DNA as the Target

A. General

During the past decades a vast amount of evidence has been obtained that points to interactions of platinum compounds with DNA in the tumor cell as the origin for cytotoxic action. The most important observations are as follows:

1. Treatment of *Escherichia coli* bacteria with *cis*-Pt leads to filamentous growth, due to hampered cell division (*1*).

2. *cis*-Pt induces lysis in lysogenic bacteria (*42*) and a good correlation between the antitumor activity and prophage induction has been found.

3. Inhibition of mainly DNA synthesis has been shown for Ehrlich ascites cells (*43*) and human HV_3 cells (*43a*), as studied by the uptake of radiolabeled DNA, RNA, and protein precursors. However, RNA and protein inhibition are still being examined (*44*).

4. The mutagenic properties of *cis*-Pt result in frameshift and base substitution mutations (*45, 45a*).

5. Bacteria (*45, 46*) and human cell lines (*47, 48*) that are DNA repair deficient are far more sensitive toward *cis*-Pt than is the DNA repair-proficient organism.

Initially these hypotheses directed almost all attention to platinum–DNA interactions in the studies concerning the working mechanism of *cis*-Pt. However, it cannot be excluded that other *cis*-Pt-induced processes at the cellular level might attribute to the ultimate cell killing. In this respect the natural immune response should be mentioned. It appears, however, that an ever-increasing amount of research is focused on the interactions of platinum compounds with DNA.

In this section a brief summary of the most important Pt—DNA interactions, including *cis*-Pt, *trans*-Pt, and other related platinum compounds, and their relevance for antitumor activity will be presented. For more detailed information the reader is referred to the several reviews that have appeared on this subject during the last 5 years (*49–53*).

B. Pt Binding to Nucleobases

Knowledge that DNA is an important target for *cis*-Pt binding raises the question of which DNA sites are preferred by the platinum antitumor compounds. This will be briefly summarized herein. Because platinum is a class B metal, it should be expected that platinum compounds show a relatively high affinity for nitrogen donor sites in DNA, i.e., the nucleobases, rather than for the phosphate–deoxyribose moiety. Indeed, Pt compounds have a high affinity for nitrogen donor atoms. At physiological pH, the potential DNA donor atoms are guanine N7, cytosine N3, and adenine N1 and N7. No coordination to other atoms has been observed at physiological pH (*51a*) (see Fig. 4). In fact, early competition experiments have shown that platinum binding to DNA monomers is largely determined by kinetic factors, and these strongly favor guanine N7 binding (*50*).

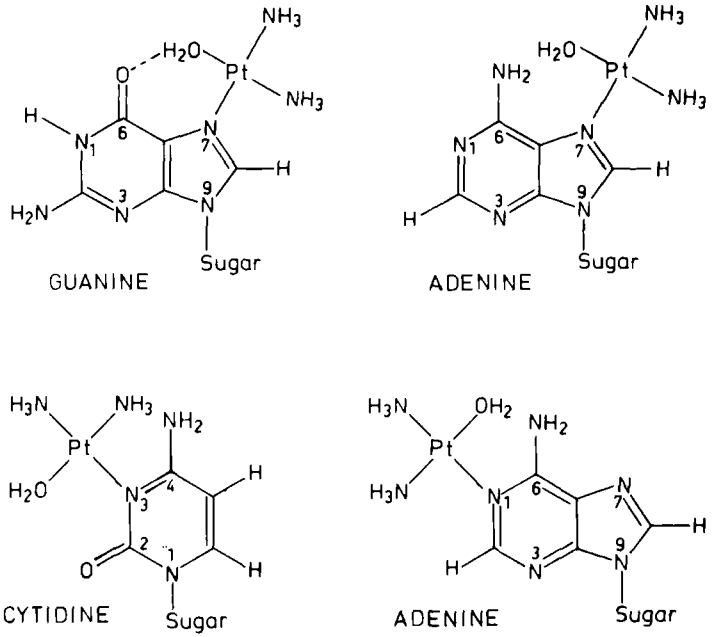


FIG. 4. Schematic structure of some nucleobases and their adducts with platinum amines.

Proton NMR has been widely applied and has been proved to be a powerful method to determine the details of these binding sites. The major conclusions can be summarized as follows:

1. The resonances of nucleobase protons that are present in the near vicinity of the platinated site are strongly shifted downfield, i.e., the purine H8 signal after N7 platination.

2. Because the chemical shifts of the nonexchangeable nucleobase proton signals are sensitive to (de)protonations of the aromatic structure, the absence of certain "protonation shifts," or alterations in the expected pK_a values, can give valuable information about the sites where platinum is bound. So, in N7-platinated guanine, no N7 protonation shift around pH 2 is observed, whereas the pK_a of the N1 protonation decreases from 9.5 to 8.5 (*54*).

3. A characteristic $^3J(^{195}Pt-^1H)$ coupling, manifested as "satellites" around the H8 resonance, is often observed in proton NMR spectra of N7-platinated guanine residues, when recorded at low magnetic fields, for instance, 60 MHz. This coupling, caused by ^{195}Pt (34% abundance),

disappears at higher fields due to efficient chemical shift anisotropy (CSA) relaxation (*54a*). Interestingly, this coupling remains visible in the spectra of Pt(IV)–nucleobase compounds when recorded at higher fields. This observation can be used as a tool to discriminate between Pt(II) and Pt(IV) adducts (*54b*).

It has also been established that in oligonucleotides, from di- to dodecanucleotides, reactions with *cis*-Pt or related Pt(II) compounds yield largely guanine N7 adducts (*55*). Several detailed NMR structures for such adducts have been reported in the last 5 years. The major conclusions are compatible with those described below for DNA, and will be reviewed in Section III,D.

C. Binding of Pt to DNA

A very interesting aspect of platinum–DNA interactions concerns the nature of the resulting adducts and their relative quantities. Due to the bifunctional nature of *cis*-Pt, several types of adducts in the DNA can be expected to be formed, to be distinguished in (1) interstrand chelates (binding of two nucleobases that are each positioned in one of the complementary DNA strands), (2) intrastrand chelates (binding of two nucleobases within the same DNA strands), (3) intrabase chelates (binding to two different atoms in one base), and (4) DNA–protein cross-links.

Once platinum is bound to DNA, the products appear to be very stable. Only strong nucleophiles such as thiourea (*56*) and cyanide (*57*) can relatively rapidly reverse the Pt–DNA bond. The binding of *cis*-Pt to DNA perturbs the DNA structure, which results in a decrease in melting temperature (*58, 59*), shortening (*60*), unwinding (*61*), and local denaturation (*62*) of the DNA helix. However, the degree of these distortions strongly depends on the used platinum levels. The distortion of the DNA structure as a result of the binding of one *cis*-Pt molecule proved to be only small (a few base pairs) (*57*).

A useful method (*63*) to study the binding positions of *cis*-Pt in DNA is based on the digestion of the high-molecular platinated DNA by enzymes, resulting in mononucleotides and platinum-containing mono- and dinucleotides. After degradation, the digestion mixture can be separated on the basis of charge by anion-exchange chromatography, and subsequently the platinated fragments can be identified and quantitated, e.g., by spectroscopic methods or immunochemical techniques. In this way, four reaction products of *cis*-Pt with salmon sperm DNA have been isolated, together comprising at least 90% of the platinum

input (*63*). The structures of these adducts have been unambiguously assigned by proton NMR and Pt analyses (atomic absorption spectroscopy; AAS). In all cases, *cis*-Pt is linked to the N7 atom of guanine or adenine, and no indications have been found for binding at adenine N1, or at cytosine N3. The quantitation results of this study have demonstrated that platinum chelates are preferably formed on neighboring guanines, the so-called GG adduct (65%). To a lesser extent, also the AG chelate (25%) was found, but surprisingly no GA chelate. Also, an adduct originating from *cis*-Pt bound to two nonneighboring guanines (10%) could be isolated as [*cis*-$Pt(NH_3)_2$(GMP-N7)$_2$]. This product is thought to originate both from interstrand chelates and so-called GNG intrastrand chelates, i.e., adducts in which *cis*-Pt is bound to two next-neighboring guanines. Finally, also a minute amount of a monofunctional adduct (5%) with guanine could be identified and quantitated. At this point it should be noted that it is not known yet which of these adducts may be the "critical lesion(s)" that lead to cell mortality. The large abundance of the GG chelate has resulted in several studies by a variety of groups (*50–55*) on this adduct.

The six different kinds of major reaction products between *cis*-Pt and DNA that have been found are depicted in Fig. 5. Two of them (4 and 6)

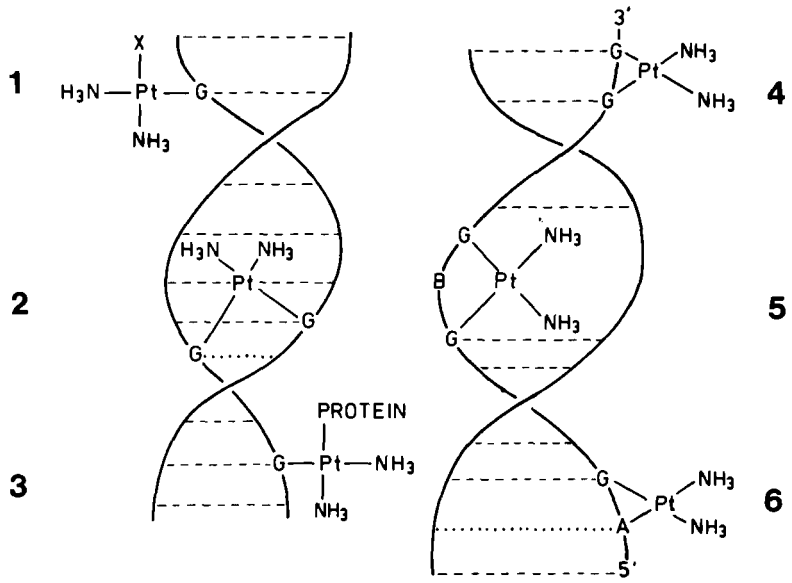


FIG. 5. A schematic representation of the known *cis*-Pt–DNA interactions. X = Cl^-, OH^-, and H_2O. See text for discussion.

are specific for *cis*-Pt and analogs. The other four can also be formed by trans isomers of platinum amine compounds. From the observation that the adducts 4 and 6 are specifically formed by *cis*-Pt and not by the inactive trans isomer, it seems likely that at least one of them is important for antitumor activity.

The intrastrand cross-link between two adjacent guanines (60–65%) (*63*) is higher than the statistically expected value of 44%, assuming a random distribution and an equal reactivity of all guanines. Therefore some kind of directing effect must be present, leading to binding of *cis*-Pt preferably to GG sequences in the DNA.

During *in vivo* studies under biologically relevant conditions, the *cis*-Pt loading of the DNA is much lower than for the above-mentioned *in vitro* studies. It has been calculated that mortality of HeLa cells occurs at an r_b value of 10^{-5} (i.e., one bound *cis*-Pt molecule per 10^5 nucleotides) (*64a*). This excludes atomic absorption spectroscopy for identification of the *in vivo* adducts. Immunochemical techniques, however, have shown to be very promising, and high sensitivity and selectivity levels have been reached. At the moment, only a few studies in which antibodies are raised against *cis*-Pt-treated DNA (*64*) or against synthetic *cis*-Pt adducts with mono- or dinucleotides are available (*64a*). With the latter method, quantitation of the different platinum–DNA adducts formed under *in vivo* conditions is possible. At the moment, femtomole (10^{-15} mol) amounts of the adducts can be detected with competitive enzyme-linked immunosorbent assay (ELISA) techniques. It has been demonstrated in this manner that the GG–Pt adduct is also the predominant adduct under *in vivo* conditions.

D. Study of Pt Binding to Oligonucleotides

As said above, only a summary of the many reported studies can be given here, and for details the reader is referred to the reviews (*49–53*) and the papers of the groups mentioned in Section III, B (*55*). A complete conformational NMR analysis of the solution structure of the adduct with a dinucleotide, i.e. [*cis*-Pt(NH_3)$_2${d(GpG)-N7(1),N7(2)}], has been available for some time (*50b*). The main features of this structure can be summarized as follows:

1. The two bases are coordinated through N7 to *cis*-Pt in a "head-to-head" orientation with a dihedral angle of about 60(20)°.

2. The deoxyribose moiety of the 5′ guanine has adopted an almost pure N-type conformation, compared to the S-conformation in free d(GpG).

3. The other conformational characteristics of d(GpG) are hardly changed upon the platination. The crystallization of *cis*-Pt adducts with oligonucleotides has been proved to be very difficult and only recently a few X-ray structures have become available (see below).

The observation that *cis*-Pt chelates preferably neighboring guanines led to studies (*55*) in which larger oligonucleotides with this sequence were reacted with the platinum compound. In addition, studies with the *trans*-Pt and with monofunctional Pt compounds have been performed. Again, only a brief summary can be given here. The distortion of DNA by *cis*-Pt appeared to be so large that up to self-complementary hexamers, no duplex formation occurs after platination. However, the GG-platinated non-selfcomplementary decanucleotide d(TCTCGGTCTC) forms a duplex with its complementary strand d(GAGACCGAGA). Detailed analysis of proton and phosphorus NMR spectra and consideration of the circular dichroism (CD) spectra led to the following conclusions (*64b*), which have been confirmed by others for related oligonucleotides (*55*).

1. The double helix is somewhat destabilized after the platination, as reflected by the decrease of the melting temperature of the duplex by 10–20°C at NMR concentrations (3 m*M*).

2. Base pair formation by hydrogen bonding seems also possible after the platination, as reflected by the appearance of iminoproton resonances. For the central GG sequence, these signals are only observed at low temperature, although shifted to lower field and broadened.

3. A careful study of the NMR spectra followed by conformational analysis suggests a rather small distortion of the double helix of this decanucleotide upon platination, which has been described as a "kink" of about 40° in the helical axis at or around the GG lesion. Later studies of Lippard (*72*) have determined the kink more accurately at 33°, with an unwinding at 13°.

4. Comparison of CD spectra and ^{31}P NMR spectra of both platinated DNA from several sources and the platinated ds decanucleotide, strongly suggets similar distortions in both cases. Molecular dynamics calculations by Kozelka *et al.* (*64c*), based on the above-mentioned decanucleotide, indicated that this kinklike structure can indeed exist. Just as in the crystals of the *cis*-Pt adduct of d(pGpG), an NH_3—OPO_3 hydrogen bond appears to be present in the calculated ds decanucleotide structure.

The recognition that the DNA structure distortion, resulting from platinum binding at GG, is rather small and localized and that the binding does not severely unwind the double helix, has directed re-

search to detailed three-dimensional studies of small GG-containing DNA fragments.

Lippard (*64d*) succeeded in solving the X-ray structure of the *cis*-Pt adduct with d(pGpG). It turned out that the solid-state geometry of this compound is roughly the same compared to the above-mentioned solution structure of the GG adduct (*50, 50b*). The role of the 5′-phosphate group seems to be a stabilizing one, i.e., it is involved in a hydrogen bond with an NH_3 ligand of platinum. This could be an important explanation for the observation that platinum antitumor drugs need an acid N—H group to donate a hydrogen bond to a phosphate and/or a guanosine—O6. The phosphate–ammonia interaction could induce and/or stabilize DNA distortions, thereby interfering with the replication process.

A very similar structure was found subsequently for the adduct with d(CpGpG) (*64e*). The solution structure of the *cis*-Pt adduct of d(CpGpG) has been determined earlier by NMR (*50b*), and shows that in spite of the *cis*-Pt moiety, the cytosine base can still stack rather well on the central platinated G, whereas the structure of the platinated GG part is similar to the above-mentioned structure of the platinated d(GpG). Subsequently the crystal structure (*64e*) of the d(CpGpG) adduct could be determined by using X-ray diffraction. In the three independent molecules present in the crystal, no stacking interactions of the cytosine on the central guanine are present. On the other hand, extensive intermolecular stacking interactions and hydrogen bonding in the solid state would explain this discrepancy with the solution structure. Apparently, in the solid state the intermolecular forces are stronger than the stacking interactions in solution. We now have to wait till detailed three-dimensional structures, based on X-ray diffraction, will become available on platinated, double-stranded oligonucleotides.

E. Binding of Other Pt Compounds to Nucleic Acids

Many other active and inactive compounds have been reacted with oligonucleotides and with DNA. It appears that the compounds with a structure related to *cis*-Pt bind to DNA, forming very similar products, although the kinetics differ (*64f*).

The two classes of antitumor-active compounds [Pt(diam)(R′R″SO)Cl](NO_3) (*23*) and [*cis*-PT(NH_3)$_2$(N-het)Cl]Cl(*24*) are in principle "monofunctional" platinum compounds (see also Section I and Fig. 3) and their antitumor activity cannot be simply explained by the formation of products such as 4 and 6 in Fig. 5, especially because other

"monofunctional" cationic complexes such as $[Pt(NH_3)_3Cl]^+$(*65*) and $[Pt(dien)Cl]^+$(*14*) were found to be antitumor inactive. These complexes are often used to model the first binding step of platinum antitumor compounds to DNA (*66–68*).

$$[Pt(diam)(R'R''SO)Cl] \xrightarrow[-Cl^-]{+DNA} [Pt(diam)(R'R''SO)(DNA)] \xrightarrow[-R'R''SO]{} [Pt(diam)(DNA)] \quad (8)$$

$$[Pt(diam)(R'R''SO)Cl] \xrightarrow[-R'R''SO]{+DNA} [Pt(diam)(DNA)Cl] \xrightarrow[-Cl^-]{} [Pt(diam)(DNA)] \quad (9)$$

For $[Pt(diam)(R'R''SO)Cl]^+$, the formation of a sulfoxide–Pt–DNA [Eq. (8)] or a chloride–Pt–DNA intermediate [Eq. (9)] with subsequent activation by *in vivo* displacement of sulfoxide or chloride, respectively, by a nucleic acid base (i.e., guanine) can explain the antitumor activity (*23*). The inertness of R'R''SO with respect to displacement by both chloride and water precludes the possibility of these complexes acting by simple loss of the sulfoxide ligand (*23*). Therefore the mechanism presented in Eq. (8) is the most likely one.

Recent ^1H NMR results of binding studies of the relatively simple compound $[Pt(en)(Me_2SO)Cl]Cl$ with the dinucleotide d(GpG) and with 5'-GMP are supportive for such a mechanism (*69*). Initially, relatively long-lived species of the type $[Pt(en)(Me_2SO)G]$ are formed. Eventually, Me_2SO hydrolysis occurs while forming [Pt(en){d(GpG)-N7(1),N7(2)}] and $[Pt(en)(5'GMP\text{-}N7)_2]$, the same as formed with the classical antitumor drug $[Pt(en)Cl_2]$. Recently, a novel synthesis has been described to prepare symmetrical and unsymmetrical (malonato) platinum(II) complexes (*70*). The intermediate species in this synthesis {[*cis*-Pt(DACH)(Me_2SO)(CBDCA)]; Fig. 3, compound C} might, in view of the above, exhibit interesting antitumor activities.

For $[cis\text{-}Pt(NH_3)_2(N\text{-het})Cl]^+$ compounds an ammonia-loss pathway, as a result of the trans labilizing effect of a N-het, to achieve didentate binding to DNA has been considered as a possible mechanism of action (*24*). It is equally possible that $[cis\text{-}Pt(NH_3)_2(N\text{-het})Cl]^+$ is monofunctionally bound to DNA, followed by a subsequent interaction of the N-het and DNA. Such an intercalative interaction must be located in a way to produce a nonrepairable lesion of DNA (*24*). It should be stated that these mechanisms of action form part of the discussion and are not definite hypotheses.

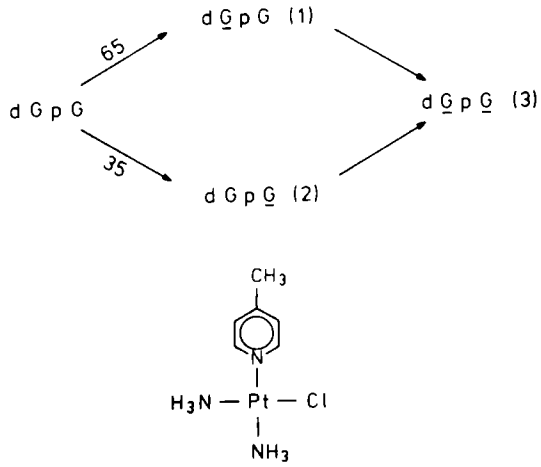
Reactions of the triamine complexes such as $[cis\text{-}Pt(NH_3)_2(4\text{-mepy})Cl]Cl$ with d(GpG) proved that there is no release of amine ligands and therefore the formation of bifunctional adducts, comparable to those induced by *cis*-Pt, is highly unlikely (*71, 72*). Only two mononu-

clear complexes, [*cis*-Pt(NH_3)$_2$(4-mepy){d(GpG)-N7(1)}] (**1**) and [*cis*-Pt(NH_3)$_2$(4-mepy){d(GpG)-N7(2)}] (**2**), and one dinuclear complex, [*cis*-Pt(NH_3)$_2$(4-mepy)]$_2${μ-d(GpG)-N7(1),N7(2)}] (**3**), are formed (see Scheme 1 (*71*). Even extreme conditions, such as the addition of nucleophilic sulfur-containing molecules (*71*) or raising the temperature (*72*), did not induce the formation of a chelate. In addition, the results (*71*) of bacterial survival and mutagenesis experiments with *E. coli* strains and data (*72*) obtained with monoclonal antibodies that bind to *cis*-Pt–DNA lesions showed that the *in vivo* formation of bifunctional adducts in DNA and a mechanism of both GN7 binding and intercalation are unlikely. Whether the Pt–DNA binding in general or other mechanisms are important for the antitumor activity of [*cis*-Pt(NH_3)$_2$(N-het)Cl]Cl remains unclear and is a subject of further studies. The fact that these compounds inhibit DNA replication at individual guanine residues is supportive for the importance of Pt–DNA binding (*72*).

IV. Platinum–Sulfur Interactions

A. General

Since the discovery of *cis*-Pt as an antitumor drug, the research on the mechanism of action has mainly been focused on the interactions with DNA, as summarized in the preceding section. Although such interac-



SCHEME 1. Summary of the two-step reaction of d(GpG) with [*cis*-Pt(NH_3)$_2$(4-mepy)Cl]Cl. The indicated values denote relative amounts (%).

tions are generally accepted to be ultimately responsible for antitumor activity, there are many other important biomolecules that can react with platinum amine compounds. Especially sulfur-containing molecules have a high affinity for platinum (*73*). Possible reactive biomolecules are cysteine, methionine, *S*-adenosyl-L-homocysteine, *S*-adenosyl-L-methionine, glutathione, metallothionein, and other proteins. Only in the last decade has information about (bio)chemically and medically interesting Pt–sulfur interactions become available. In general, interactions of platinum antitumor compounds with sulfur-containing biomolecules are considered to have an overall negative effect on the antitumor activity. Such interactions can be responsible for inactivation of Pt(II) species, for development of resistance, and for toxic side effects such as nephrotoxicity. In this section these interactions and their relevance in the mechanism of action of *cis*-Pt, as an antitumor drug, will be summarized. In addition, the mechanism of action of some nephrotoxicity-reducing agents, i.e., those with sulfur, such as sodium diethyldithiocarbamate (Naddtc) and sodium thiosulfate (STS), will be discussed.

B. Inactivation of Platinum Amine Compounds

The reaction of sulfur-containing biomolecules with platinum antitumor compounds, thereby preventing binding to the critical DNA target, is a possible mechanism of inactivation and is supported by numerous studies. Thus, glutathione (GSH, a cysteine-containing tripeptide; see also Fig. 6), which is the predominant intracellular thiol and is present in concentrations varying from 0.5 to 10 m*M*, is able to inhibit the reaction of DNA with [Pt(en)Cl_2] (*74*) and with *cis*-Pt (*75, 76*). It has also been observed that the presence of cysteine can inhibit the reaction between *cis*-Pt and d-Guo (*77*). Furthermore, the antitumor activity of *cis*-Pt was proved to be inhibited by coadministered methionine (*78, 79*) and even a bis-adduct between *cis*-Pt and methionine has been isolated from the urine of patients (*80*).

The reactions of *cis*- and *trans*-Pt have been recently investigated with the use of ^{1}H NMR by Berners-Price and Kuchel (*81*) in intact

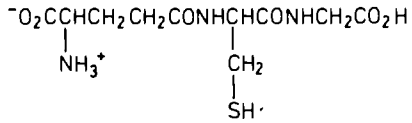


FIG. 6. Structure of glutathione.

human red blood cells. As expected, they observed that both Pt compounds react with GSH, leading to binding of the Pt species, as shown in Fig. 7. In addition to the binding to GSH, binding to hemoglobin was also observed. *trans*-Pt reacts more rapidly with GSH, which would suggest that in the case of *cis*-Pt higher percentages of the drug dose may reach the cell nucleus, before inactivation with GSH takes place. The more rapid reaction of *trans*-Pt compared to *cis*-Pt can be explained by the more labile chloride atoms in *trans*-Pt due to the trans effect; i.e., the chloride in *trans*-Pt is a better labilizer than is the amino group in *cis*-Pt (*73*).

A second mechanism of inactivation might be the reaction of sulfur-containing biomolecules with the *cis*-Pt–DNA monoadducts (product 1 in Fig. 4), which prevents those from rearranging to toxic bifunctional adducts. Supportive for such a mechanism is the observation that GSH can be cross-linked to DNA by *cis*-Pt (*41, 41a*) and $[Pt(en)Cl_2]$ (*74*), and that cysteine can be cross-linked to d-Guo by *cis*-Pt (*77*). Furthermore, *cis*-Pt–DNA monoadducts can be experimentally quenched with thiourea, which reduces drug toxicity (*82, 83*). *trans*-Pt also yields monofunctional adducts after reaction with DNA, and these rearrange somewhat slower than does *cis*-Pt into bifunctional adducts (*41, 84*), clearly for sterical reasons. The relatively long-living monofunctional adducts react efficiently with GSH and proteins (*41a, 84–86*).

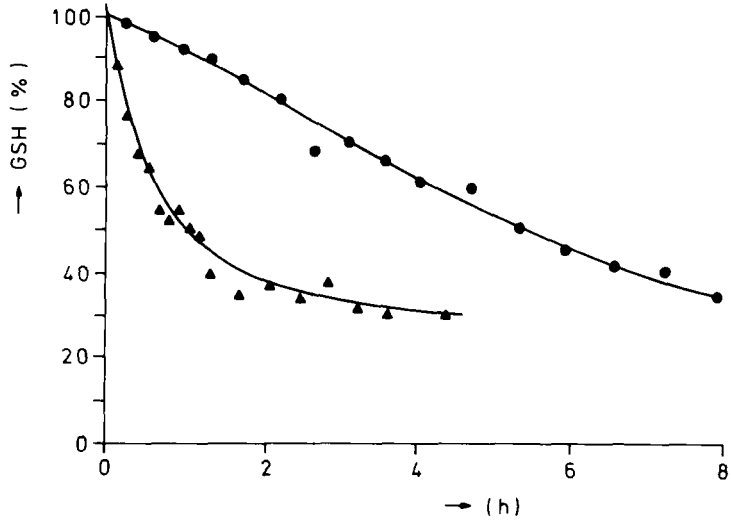


FIG. 7. Depletion of intracellular GSH on incubation at 310 K of red blood cells with *cis*-Pt (●) and *trans*-Pt (▲).

It can be stated now that in principle all S-containing biomolecules can inactivate Pt amine compounds by the two mechanisms and it is probable that the inactivation is not limited to the above described binding by GSH, methionine, and cysteine. For instance, it has been reported that high percentages of *cis*-Pt are bound to metallothionein (MT; a protein with a molecular weight of 6000–7000, containing 33% cysteine) in Ehrlich tumor cells (30%) (*87*), in *cis*-Pt-resistant cells (70%) (*88*), and in rat tissues (25–40%) (*89, 90*). In comparing *cis*-Pt and *trans*-Pt it has been shown that both inactivation mechanisms [i.e., the reaction with free Pt(II) species and with Pt(II)–DNA monoadducts] are more pronounced for *trans*-Pt, which could—at least partly—explain the reduced toxicity of this antitumor-inactive compound. This hypothesis is in agreement with studies in which especially the cytotoxicity of *trans*-Pt (*91*), but not of *cis*-Pt (*75, 91*), to a human ovarian carcinoma cell line was found to be increased markedly by depleting GSH levels.

C. Resistance to Platinum Antitumor Compounds

The clinical usefulness of *cis*-Pt is often limited by the development of resistance. The development of resistance to *cis*-Pt has been explained by several factors, including reduced drug accumulation, increased DNA repair processes, and an increase in the amount of inactivation proteins. In most *cis*-Pt-resistant tumors probably a combination of such factors plays a role. In this section, only an increase in cellular thiols in relation to resistance will be discussed. Such inactivation processes have already been discussed (Section IV,B), but attention will be directed now to the mechanism of resistance. These mechanisms are probably also of importance for the inactivation of *cis*-Pt in nonresistant tumors, though to a lesser extent.

It has been shown that many cancer cell lines that are resistant to *cis*-Pt have elevated levels of gluthathione (Table I) (*92–101*); on the other hand, depletion of GSH with buthionine sulfoxime reverses resistance only for the A2780 ovarian carcinoma cell line (*93*). Resistant cancer cell lines, which have no elevated levels of GSH, could also not be sensitized by depletion of GSH (*75, 91, 102*). These results are rather controversial and therefore the precise role of GSH in mediating *cis*-Pt resistance is still unclear at present. More research is necessary to explain the rather low increased levels of GSH with respect to the sometimes significant increase in resistance.

It has recently been shown by Kelley *et al.* (*103*) that tumor cell lines resistant to *cis*-Pt have significant elevated levels of MT (Table II). In contrast, the results of Schilder *et al.* (*104*) did not show a correlation

TABLE I

FUNCTION OF GLUTATHIONE IN *cis*-Pt RESISTANCE

Cell line	Tumor type	Increase factor in resistance	Increase factor in GSH	Ref.
A2780	Human ovarian carcinoma	14	3.2	*92,93*
P388	Mouse leukemia	24	1.2	*94*
L1210	Mouse leukemia	100	1.7	*95*
COLO 316	Human ovarian carcinoma	13	2.3	*96*
GLC_4	Human lung carcinoma	6.4	3.4	*97*
O-342	Rat ovarian carcinoma	?	1.9	*98*
ROT 68/C1	Rat ovarian carcinoma	20	1.4	*99*
RIF-1	Mouse fibrosarcoma	2.3	1.4	*100*
BE	Human colon carcinoma	5	3	*101*

between MT expression and *cis*-Pt resistance. The cell lines in Table II have been exposed to high concentrations of *cis*-Pt for long periods of time, which raises the question whether such a mechanism of development of resistance also occurs in clinically relevant situations. Again, much more work is needed to establish the exact role of MT in the development of resistance. It also remains to be determined whether long-term exposure to *cis*-Pt induces MT synthesis either directly (as $CdCl_2$ is known to do), or indirectly. Recent results showed that *cis*-Pt doubles the amount of MT in the liver and kidney of mice and it was concluded that *cis*-Pt can directly induce the synthesis of MT, although only when present in the hydrolyzed form (*105*).

TABLE II

FUNCTION OF METALLOTHIONEIN IN *cis*-Pt RESISTANCE[a]

Cell line	Tumor type	Increase factor in resistance	Increase factor in MT
SCC25	Human head and neck carcinoma	7.1	4.4
G3361	Human melanoma	6.7	2.0
SW2	Human small cell carcinoma	4.5	5.1
SL6	Human large cell carcinoma	2.5	3.4
L1210	Mouse leukemia	44	13.3

[a] From Ref. *103*.

D. Nephrotoxicity and Rescue Agents

A limitation of *cis*-Pt in its use as an antitumor drug is its concentration-dependent nephrotoxicity (*106, 107*), besides a variety of other side effects (*108*). Currently, the nephrotoxicity effects can be reduced by mannitol-induced diuresis (*109*) and hypersalination (*110*). On the basis of a similarity of histopathology of the kidney after Pt(II) or Hg(II) exposure in the rat, it has been suggested by Borch and Pleasants (*111*) that a similar mechanism might play a role in the nephrotoxicity of these metals [i.e., inactivation of enzymes by the coordination of Pt(II) or Hg(II) to thiol residues]. Supportive of this mechanism is that the total number of protein-bound sulfhydryl groups is depleted (14%) in kidneys after *cis*-Pt administration, especially in the mitochondrial fraction (*112, 113*). The enzyme adenosine triphosphatase, which is critical for kidney function, has been proposed as the site of action (*114*), although the high concentrations necessary for inhibition are unlikely to be achieved *in vivo*. Recently, it was demonstrated that the activity of gluthathione peroxidase was decreased significantly in *cis*-Pt-treated kidney mitochondria resulting in dysfunction (*115*). However, which enzymes are important in causing the nephrotoxicity is still controversial. Also, other mechanisms, not based on inactivation of enzymes, may play a role. Recently, it was postulated that the mitochondrial DNA damage induced by *cis*-Pt causes nephrotoxicity (*116*).

The affinity of sulfur for platinum complexes has led to investigations of numerous sulfur nucleophiles as inhibitors of *cis*-Pt nephrotoxicity, including Naddtc, STS, WR-2721, mesna, methionine, thiourea, cysteine, *N*-acetylcysteine, penicillamine, and GSH. Of these, Naddtc, STS, and WR-2721 are undergoing preclinical and/or clinical evaluation (Fig. 8). Some of the more promising compounds will be discussed here.

Naddtc has proved to be a very effective inhibitor of nephrotoxicity [it is also effective against bone marrow toxicity (*117–119*)] and should be administered 1–4 hours after *cis*-Pt, without interfering with the anti-

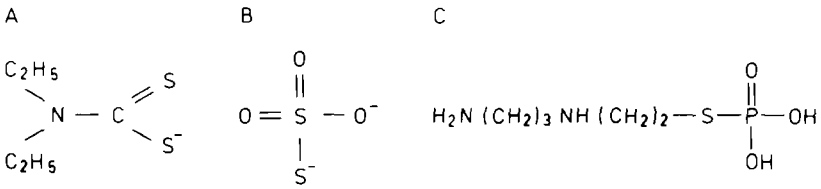


FIG. 8. Structure of the nephrotoxicity inhibitors ddtc$^-$ (A), TS$^-$ (B), and WR-2721 (C).

tumor properties of *cis*-Pt (*111, 117, 120–123*). The noninterference with the antitumor properties of *cis*-Pt is in agreement with the observation that Naddtc could not reverse Pt–DNA cross-links (*123*), except for the *cis*-Pt–adenosine 1:2 complex and the *cis*-Pt–guanosine 1:1 complex (*124*), which are considered to be not important for the antitumor activity of *cis*-Pt. Naddtc and thiourea (*vide infra*) are the only rescue agents that provide protection from nephrotoxicity when administered after *cis*-Pt, at a time when most of the reactive platinum species have been taken up by cells or are already being excreted in the urine. Although pretreatment with Naddtc is inefficient in inhibiting nephrotoxicity, probably because it inactivates *cis*-Pt, one study is known in which treatment with Naddtc 12 hours prior to *cis*-Pt was effective in protecting kidney damage (*125*). This might be related to the fact that Naddtc is a powerful inducer of MT synthesis (*126*), which could be the actual inhibitor of the nephrotoxicity (i.e., by reacting with free *cis*-Pt in the kidney). Especially the time of administration of Naddtc, i.e., after *cis*-Pt treatment, agrees with the hypothesis that Naddtc acts as a real rescue agent. Thus it reduces the nephrotoxicity by removing the platinum from certain proteins, thereby restoring the original structure of the protein (111). Evidence for the Naddtc-induced dissociation of Pt–protein adducts has been reported. Thus α_2-macroglobulin (*127*), γ-glutamyltranspeptidase (*76, 124, 128*), and fumarase (*129*) are inactivated by *cis*-Pt and reactivated by Naddtc (e.g., see the reactivation experiments with fumarase in Fig. 9). Unfortunately, only for α_2-macroglobulin is the actual interaction of the protein with *cis*-Pt known, i.e., it is cross-linked through its methionine residues (*127*).

In order to investigate what kind of Pt–enzyme interactions can be

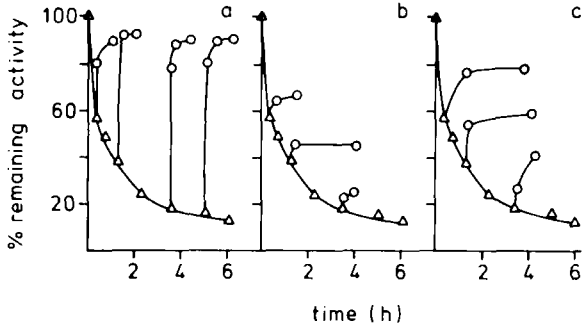
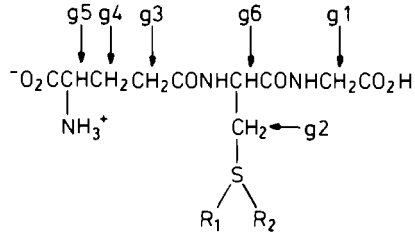


FIG. 9. Inhibition of fumarase by *cis*-Pt (△) and regeneration (○) by Naddtc (a), STS (b), and thiourea (c). The values for Naddtc and STS remained constant for 24 hours, but those for thiourea increased to 80%.

reversed, exchange reactions of model compounds with Naddtc were performed. The well-identified model compounds (*130*) (Fig. 10) can be considered to mimic Pt–cysteine and Pt–methionine adducts within a protein (*131*).

The results (*131*) have shown (Table III) that particularly the Pt–methionine type of bond in $[Pt(dien)GS\text{-}Me]^{2+}$ reacts fast with Naddtc, thereby restoring the original structure of the thioether linkage. The Pt–cysteine type of bond in $[Pt(dien)GS]^{+}$ appears to be inert toward Naddtc treatment. This is in agreement with the results of exchange reactions of Naddtc with the not well-characterized, but biologically more relevant, *cis*-Pt–methionine and —GSH system, which also points to the lability of only the Pt–methionine types of interactions (*131, 132*). The inert Pt—S bond in Pt–cysteine complexes under neutral conditions is probably the result of a very strong negatively charged Pt—S^- binding compared to rather weak neutral Pt—S binding in Pt–methionine complexes. Extrapolating the results obtained with the model adducts to a protein leads to the following hypothesis (*131*) for the mechanism of reducing the nephrotoxicity by Naddtc: Naddtc would only reduce the nephrotoxicity when the Pt binding to proteins is caused by Pt coordination to methionine residues and not to cysteine residues. This is in contrast with the initial hypothesis (*111*), which suggests that Naddtc reverts the Pt–cysteine type bonds in proteins.



Model Adduct	R_1	R_2
Pt-methionine	CH_3	$Pt(dien)^{2+}$
	$Pt(dien)^{2+}$	$Pt(dien)^{2+}$
Pt-cysteine	$Pt(dien)^{2+}$	-------

FIG. 10. Schematic representation of the products formed between GSH, GS-Me, and [Pt(dien)Cl]Cl. The complexes are considered as models for Pt–protein interactions.

TABLE III

HALF-LIVES AND PRODUCTS OF EXCHANGE REACTIONS BETWEEN NADDTC, THIOUREA, OR STS AND PLATINATED SULFUR COMPOUNDS[a]

Compound	Naddtc	Thiourea	STS	Product
$[Pt(dien)GS]^+$	—	—	—	No reactions
$[Pt(dien)GS\text{-}Me]^{2+}$	<2 minutes	30 min	<2 minutes	GS-Me
$[\{Pt(dien)\}_2GS]^{3+}$	40 minutes	7 hours	20 minutes	$[Pt(dien)GS]^+$

[a] From Ref. *131*.

Naddtc does not react with all the platinated proteins, as was observed by Hegedus *et al.* (*133*). Therefore, the nephrotoxicity of *cis*-Pt is likely to be the result of inactivation of certain enzymes through binding at cysteines and/or methionines and Naddtc can reduce the nephrotoxicity only by reversing the Pt—S bonds of the methionine type.

Unfortunately, Naddtc is known to cause severe side effects, including burning of the mouth, chest tightness, and extreme anxiety (*134*). However, recently it has been demonstrated that the use of other dithiocarbamates, such as sodium di(hydroxyethyl)dithiocarbamate (Fig. 11), possess certain promising advantages (*125, 135, 136*). The polar groups on these derivatives of Naddtc can be expected to result in *cis*-Pt complexes with a character different than those of complexes formed with Naddtc (*136*). For example, the solubility will be much higher, which may result in lower toxic side effects.

Thiourea probably acts in a manner comparable to that of Naddtc and should also be administered after *cis*-Pt treatment (*78*). Like Naddtc, thiourea is able to remove platinum from platinated enzymes, such as leucine aminopeptidase (*76, 128*), γ-glutamyltranspeptidase (*76, 128*), and fumarase (*129*) (Fig. 9), and from Pt–methionine model adducts (Table III) (*131*). However, thiourea appears to be less useful as an inhibitor of nephrotoxicity; it also reacts quite rapidly with platinum–DNA cross-links (*56*).

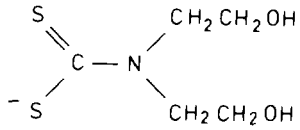


FIG. 11. Structure of the nephrotoxicity inhibitor di(hydroxyethyl)dithiocarbamate.

The rescue agent sodium thiosulfate (STS) (Fig. 8), which as such is nontoxic (*137*), should be injected during the period 1 hour before and 0.5 hour after *cis*-Pt treatment (*138*). The optimal protocol in fact uses STS intravenously (i.v.) in conjunction with intraperitoneal (i.p.) *cis*-Pt (*139–141*). Concurrent injection of STS and *cis*-Pt, i.p. (*138*) or i.v. (*142*), partly reduces the antitumor activity, probably by inactivation of *cis*-Pt. A likely explanation for the nephrotoxicity protecting effect is that STS is concentrated extensively in the kidney (*137*), where it has been proved to react rapidly with *cis*-Pt (*143*).

Numerous studies have shown that protein-bound *cis*-Pt cannot be released by STS (*76, 128, 129, 143*) (Fig. 9), although STS is able to break the Pt—S bond of the methionine type in the model system (Table III) (*131*). Therefore, this model system, does not mimic enzymes in every detail for the reaction with STS. This difference might originate from the 2^- charge of STS compared to the 1^- charge in Naddtc (*129*), keeping STS more separated from the active site.

Thus, though Naddtc acts as a "true" rescue agent, i.e., by reversing Pt–biomolecule interactions, STS most likely acts by local inactivation of *cis*-Pt and its concentration in the kidney. This may also explain why STS is not effective when administered after *cis*-Pt treatment.

The mechanism by which the more recently used WR-2721 (Fig. 8) reduces nephrotoxicity is not very well understood. WR-2721 protects against nephrotoxicity when administered just prior to *cis*-Pt (*144, 145*) and it is known that WR-2721 is preferentially taken up by normal cells and not by tumor cells (*146*). Recently, it was concluded that the uncharged form of the dephosphorylated WR-2721 (known as WE-1065) is the actual species taken up by both normal and tumor cells (*147*). It has been proposed that the conversion of WR-2721 to WR-1065 is slower in tumors, compared with normal tissues (*147*), possibly because tumors generally have lower levels of alkaline phosphatase (*148*). Furthermore, it has been proposed (*147*) that once formed, WR-1065 will have a decreased uptake rate in tumors, probably as a consequence of their lower pH (*149*) as compared with normal tissues; i.e., the neutral form of WR-1065 will only constitute 0.1% of the total drug present at pH 7 and 1% of the total at pH 8. The reactive WR-1065 is likely to bind directly to *cis*-Pt, thereby preventing side reactions of *cis*-Pt.

Pretreatment of rat with GSH also reduces kidney toxicity, which is particularly attractive because GSH is nontoxic and does not interfere with the antitumor effectiveness of *cis*-Pt (*150–153*). It has been proved that extracellular GSH accumulates in the kidney (*154*) and therefore it seems probable that the inactivation of *cis*-Pt also takes place in the

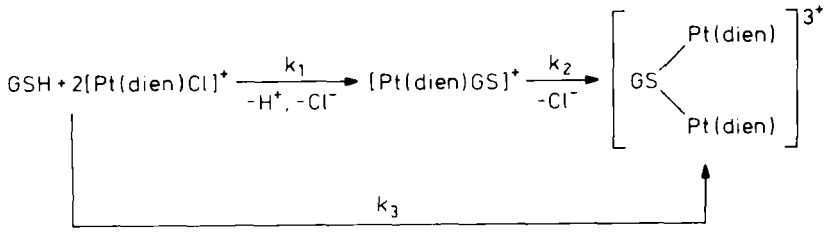
kidney. Supportive for such a protective role is that depletion of GSH levels by buthionine sulfoxime or diethylmaleate has been shown (*155, 156*) to increase the nephrotoxicity of *cis*-Pt. A recently reported observation by Mayer *et al.* (*157*) that depletion of GSH levels by buthionine sulfoxime reduces the *cis*-Pt-induced nephrotoxicity is, apparently, in contradiction to these results. A possible explanation may be that the buthionine sulfoxime-induced protection is mediated by a mechanism that is independent of GSH depletion (*157*).

Finally, it should be mentioned that a new interesting method has been developed to protect cells from *cis*-Pt nephrotoxicity. Pretreatment of mice by administration of bismuth salts induces MT synthesis, especially in the kidney, but not in the tumor (*158*). This induction of MT was found to decrease the renal toxicity of *cis*-Pt, whereas the antitumor activity was not affected (*158, 159*); these observations can be rationalized by assuming that *cis*-Pt binds to MT in the kidney, as has been previously observed by other investigators (*89, 90*).

E. Rate-Determining Step of Pt—S Binding

It has now been generally accepted that the hydrolysis of the chloro ligand is the rate-determining step in the reaction of *cis*-Pt with DNA (*40*). Concerning the rate-determining step of platinum amine compounds with sulfur-containing biomolecules, the available data are quite controversial. It has been reported that the chloro hydrolysis is the rate-determining step in reactions of *cis*-Pt with leucine aminopeptidase (*76*), with γ-glutamyl transpeptidase (*76, 124*), and also with albumin (*160*). However, it has also been suggested that there may be a direct binding to proteins without prior aquation (*161*), and this has been observed with cysteine (*162*), GSH (*75, 162*), adenosine triphosphatase (*76*), and with MT (*163*).

To investigate the kinetics in more detail, the reaction rates of a simple pair of model compounds, [Pt(dien)X] [(X = Cl^-, H_2O) with GSH, G*S*-Me and 5′-GMP have been investigated and compared (*164*). The reaction products with GSH and G*S*-Me are shown in Fig. 10; an overview of the reactions between [Pt(dien)Cl] and GSH is presented in Scheme 2. These products are the first well-identified complexes between S-containing biomolecules and platinum amine compounds (*130*) and therefore are ideally suited as model compounds for kinetic studies. The results of the reactions are summarized in Table IV. In agreement with the above-mentioned hypothesis, the chloride hydrolysis is the rate-determining step in the reaction of $[Pt(dien)Cl]^+$ with 5-GMP,



SCHEME 2. Overview of the reactions between [Pt(dien)Cl]Cl with GSH.

forming [Pt(dien)(5′-GMP-N7)]. This reaction can be completely inhibited in saturated NaCl. On the contrary, the reaction of Pt(dien)X with GSH and GS-Me is second order in Pt and S and the rate constant is nearly independent of the Cl^- concentration. In fact, the slightly slower reaction at higher [Cl^-] indicates that only a small fraction proceeds through the hydrolysis pathway.

These results are clear evidence that sulfur reacts mainly directly with Pt amine compounds, substituting Cl^-, without prior aquation. As is evident from Table IV, the hydrolyzed species $[Pt(dien)(H_2O)]^{2+}$ will almost selectively react with 5′-GMP (3.6 M^{-1} sec^{-1} versus 0.51 M^{-1} sec^{-1} and 0.18 M^{-1} sec^{-1}), whereas the chloro species $[Pt(dien)Cl]^+$ will only react with sulfur. This information is of extreme importance in the strategy of the development of new Pt drugs. If it would be possible to develop a compound with structural properties such that the direct attack by sulfur is inhibited, but with a similar rate of chloro hydrolysis compared to *cis*-Pt, this would lead to compounds with improved antitumor properties and lower toxicities. The data depicted in Table IV were obtained at pH 5. However, it has been proved that GS^- reacts remark-

TABLE IV

RATE CONSTANTS FOR THE FORMATION OF THE 1:1 COMPOUNDS FOR 5′-GMP AND GS-Me AND OF THE 2:1 COMPOUND FOR GSH (pH 5)[a]

Compound	5′-GMP	GS-Me	GSH (k_3)
$[Pt(dien)H_2O]^{2+}$	3.6 M^{-1} sec^{-1}	0.51 M^{-1} sec^{-1}	0.18 M^{-1} sec^{-1}
$[Pt(dien)Cl]^+$	6.2 × 10^{-5} sec^{-1}	0.03 M^{-1} sec^{-1}	0.006 M^{-1} sec^{-1}

[a] From Ref. 164.

ably faster than GSH does with [Pt(dien)Cl]Cl (*130*), which is indicative for a slightly higher reactivity of glutathione at physiological pH. This would suggest a less effective mechanism of inactivation of *cis*-Pt in tumors, as these generally have a lower pH compared to normal tissues (*149*).

F. Reactivation of Pt(II) Compounds with Antitumor Activity

The high affinity of many platinum compounds for sulfur and the availability of many sulfur-containing biomolecules have raised the question whether Pt–sulfur biomolecule interactions could serve as a drug reservoir for platination at DNA, necessary for the antitumor activity of *cis*-Pt. Two reaction paths are possible, i.e., spontaneous release of plantinum from the sulfur, or nucleophilic displacement of platinum from sulfur by guanine (N7), for example. At the moment, there is no real evidence for the existence of such reactivation mechanisms. In fact, it has been reported that Pt–protein interactions in the plasma (albumin) are not reversible under normal conditions (*161*, *165*). Further, a mixture of *cis*-Pt–methionine products does not show antitumor properties (*166*), indicating no induced platination of DNA. More research is required to investigate the existence of a reactivation mechanism. However, it is predicted that if such a reactivation phenomenon is operational, the most likely candidate is the labile Pt–methionine bond, as has been shown by its rapid reaction with Naddtc, STS, and thiourea (*vide supra*) (*131*).

G. Reduction of Pt(IV) Compounds Exhibiting Antitumor Activity

As was indicated in Section I, Pt(IV) complexes are most likely (*in vivo*) reduced to the corresponding active Pt(II) complexes. Evidence for such a mechanism comes from the fact that Pt(IV) compounds only bind to 5′-GMP in the presence of ascorbic acid (*19*). Other possible reducing agents are sulfhydryl-containing biomolecules, of which GSH is the most abundant compound in a cell. Indeed, it has been shown that in the presence of GSH, tetraplatin ([Pt(DACH)Cl_4]) reacts readily with DNA (*167*). At high concentrations of GSH, inhibition of DNA binding was observed due to a "simple" inactivation reaction between GSH and the formed Pt(II) species (*167*). In accordance with these observations, [*cis*-Pt(*i*-PrNH_2)$_2$$Cl_2$] has been identified as one of the metabolites of the corresponding Pt(IV) complex (*18*).

H. Reaction Products of Pt–Amine Compounds and Sulfur-Containing Biomolecules

In the last 5 years there has been a growing interest in the Pt—S interactions from a chemical point of view. The main reason for this is to understand the exact role (*vide supra*) of such interactions in the mechanism of the antitumor activity of *cis*-Pt. In this section the currently known products between Pt amine compounds and sulfur-containing biomolecules will be evaluated, with special attention to *cis*-Pt and the antitumor-inactive complexes *trans*-Pt and [Pt(dien)Cl]Cl. [Pt(dien)Cl]Cl is monofunctional in nature and is often used to model the first binding step of *cis*)-Pt (*66*). In addition, stable complexes can be expected (originating from the chelate effect of dien), whereas *cis*-Pt often gives complicated mixtures of products with all kinds of degradation products (*vide infra*).

Numerous *in vitro* studies have reported that both *trans*-Pt and *cis*-Pt bind to enzymes and proteins (*76, 124, 128, 129, 168–179*). In all these studies it has been suggested that cysteines and methionines are the binding sites and that, where reported, in general, *trans*-Pt reacts more readily (see also the above-described reaction with GSH) than *cis*-Pt. Only ribonucleotide reductase (*179*) appears to be an exception. Only in a few studies the actual binding site has been investigated. For thymidylate synthetase (*169*), albumin (*173, 177*), and ribonucleotide reductase (*179*), the reaction with *cis*-Pt takes place at cysteines, whereas for α_2-macroglobulin (*175, 177*) and α_1-antitrypsin (*176, 177*) the reactions take place at methionines. In general, high ratios of Pt:protein are required (at least >100) in order to observe detectable amounts of binding within a reasonable time. The presence of such interactions *in vivo* is not known. However, *cis*-Pt and [Pt(en)Cl_2] are known to react *in vivo* with plasma proteins (*165, 180, 181*), which makes it reasonable that reaction takes place, at least in those cases with albumin, α_2-macroglobulin, and/or α_1-antitrypsin.

A recent study by Bongers *et al.* (*182*) describes the interaction of MT with an excess of K_2PtCl_4. A product is formed containing 7 mol Pt/mol MT (at neutral pH) with coordination only through sulfur. Upon reaction of [*cis*-Pt(Am)$_2Cl_2$] with Mt, release of the amine ligands has been observed (*163, 182*). Therefore, products similar to those formed with K_2PtCl_4 can be expected. Such release of amine ligands upon coordination of [*cis*-Pt(Am)$_2Cl_2$] to methionine (*80, 183–186*), cysteine (*187, 188*), GSH (*76, 187–189*), and proteins (*190*) have already been observed and is rationalized by the large trans effect of a coordinated

sulfur atom, which labilizes the amine ligand trans to the sulfur (*73*). For this reason, amine release upon coordination of sulfur to [*trans*-$Pt(Am)_2Cl_2$] is not expected. It has even been speculated that the differing biological activities of *cis*-Pt and *trans*-Pt might be related to S-induced amine release only from *cis*-Pt (*190*).

A number of investigations of the reaction of *cis*-Pt with GSH have been published (*76, 187–189, 191*), but due to the complexity of the system, i.e., because of amine release, the reaction products still have not been characterized unambiguously. It has been suggested that [$Pt(GS)_2$] (*187*) with coordination via S and dehydronated peptide N atoms, or [*cis*-$PT(NH_3)_2(GS)(H_2O)$] (*191*), is formed. On the other hand, it has been proved recently that eventually a polymeric structure is formed with formula $[Pt(GS)_2]_n$ (*76, 188, 189*), involving loss of NH_3. Combining the results of the two most detailed studies (*188, 189*), it is likely that initially intermediate species such as [*cis*-$Pt(NH_3)_2(GS)Cl$] and [$Pt_2(NH_3)_4(GS)_2$] (see Fig. 12) can indeed be formed. These unstable products lose NH_3 upon standing, eventually forming the polymeric $[Pt(GS)_2]_n$ with coordination exclusively via the S atom, but with several different Pt—S and Pt—S—Pt environments.

The reaction of *trans*-Pt with two equivalents of GSH results in a monomeric complex in which two GS^- units are coordinated via the cys-S atom [Eq. (10)] (*76, 131, 189*). The unstable intermediate species, [*trans*-$Pt(NH_3)_2(GS)Cl$] and [*trans*-$\{Pt(NH_3)_2Cl\}_2GS$], could also be detected by Berners-Price (*189*). The reaction of $[Pt(NH_3)_3Cl]Cl$ with two equivalents of GS^- initially yields $[Pt(NH_3)_3GS]$, which reacts further, eventually forming [*trans*-$Pt(NH_3)_2(GS)_2$] and free NH_3 [Eq. (11) (*131*). This is rationalized by the large trans effect of the coordinated sulfur and the presence of a second nucleophilic sulfur. Therefore, the amine ligand is easily substituted by a second GS^- unit.

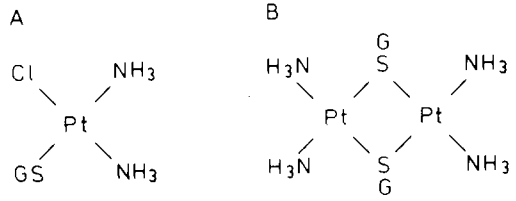


FIG. 12. Structure of the intermediate species [cis-$Pt(NH_3)_2(GS)Cl$] (A) and [$Pt_2(NH_3)_4(GS)_2$] (B) formed during reaction of *cis*-Pt with GSH.

$$[\textit{trans}\text{-}Pt(NH_3)_2Cl_2] \xrightarrow[-\,Cl^-]{+\,GS^-} [\textit{trans}\text{-}Pt(NH_3)_2(GS)Cl] \xrightarrow[-\,Cl^-]{+\,GS^-} [\textit{trans}\text{-}Pt(NH_3)_2(GS)_2] \quad (10)$$

$$[Pt(NH_3)_3Cl]+ \xrightarrow[-\,Cl]{+\,GS^-} [Pt(NH_3)_3GS]^+ \xrightarrow[-\,NH_3]{+\,GS^-} [\textit{trans}\text{-}Pt(NH_3)_2(GS_2] \quad (11)$$

For the complexes formed between GSH and [Pt(dien)Cl]Cl, the reader is referred to Fig. 10 and Scheme 2. In contrast with *cis*-Pt and $[Pt(NH_3)_3Cl]Cl$, no amine release is observed, which is rationalized by the strong chelate effect of the dien ring.

Cysteine yields a similar complex with *cis*-Pt, as shown in Fig. 12B (*188*). Other species that have been detected are [*cis*-$Pt(NH_3)_2$(Cys-S_2] and [*cis*-$Pt(NH_3)_2$(Cys-*N*,*S*)] (see Fig. 13) (*188*).

A number of complexes have been reported as products from the reaction of *cis*-Pt and methionine, i.e., [*cis*-$Pt(NH_3)_2$(Met-*S*)$_2$] (similar to that shown in Fig. 13A) (*185*), [*cis*-$Pt(NH_3)_2$(Met-*N*,*S*)] (similar to that shown in Fig. 13B) (*183, 185*), [*trans*-$Pt(NH_3)$(Met-*S*)(Met-*N*,*S*)] (Fig. 14A) (*183*), and [*trans*-Pt(Met-*N*,*S*)$_2$] (Fig. 14B) (*80, 184*). From these, [*cis*-$Pt(NH_3)_2$(Met-*N*,*S*)] and [*cis*-$Pt(NH_3)_2$(Met-*S*)$_2$] lose NH_3 on standing (*185*). Methionine yields a similar complex after reaction with *trans*-Pt, as formed between GSH and *trans*-Pt (i.e., [*trans*-$Pt(NH_3)_2$(Met-*S*)$_2$] (*192*).

The reaction of [Pt(dien)Cl]Cl with *S*-adenosyl-L-homocysteine (SAH, a biologically relevant molecule, as it is the coproduct of the methyl transfer reaction by *S*-adenosyl-L-methionine; see Fig. 15) results in a mixture of complexes (*193*), i.e., the mononuclear complex (1) $[Pt(dien)(SAH\text{-}S)]^{2+}$, with platination of SAH at the sulfur atom, the mononuclear complex (2) $[Pt(dien)(SAH\text{-}N)]^{+}$, which has a $Pt(dien)^{2+}$ unit coordinated to the amino group of the homocysteine unit, and the dinuclear complex (3) $[\{Pt(dien)\}_2(SAH\text{-}S,N)]^{3+}$, which has a $Pt(dien)^{2+}$ unit coordinated to the sulfur atom as well as a $Pt(dien)^{2+}$

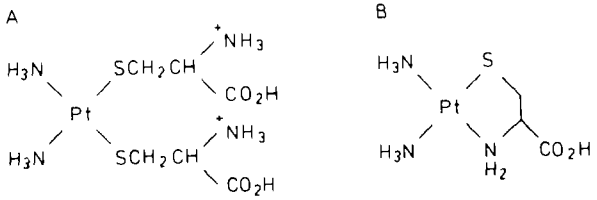


FIG. 13. Structure of [*cis*-$Pt(NH_3)_2$(Cys-*S*)$_2$] (A) and [*cis*-$Pt(NH_3)_2$(cys-*N*,*S*)] (B) formed during reaction of *cis*-Pt with cysteine.

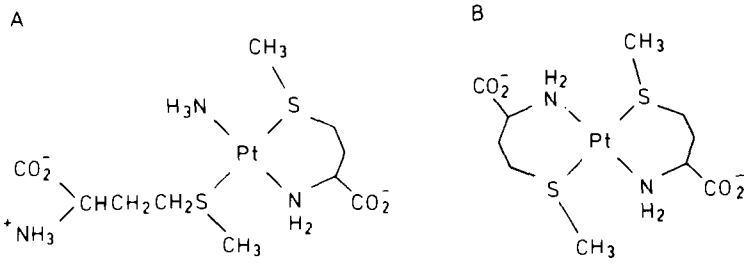


FIG. 14. Structure of [*trans*-Pt(NH_3)(Met-*S*)(Met-*N*,*S*)] (A) and [*trans*-Pt(Met-*N*,*S*)$_2$] (B) formed during reaction of *cis*-Pt with methionine.

coordinated to the amino group of the cysteine unit. The formation of these complexes and their interconversions has been depicted schematically in Fig. 16. As can be seen, the Pt–methionine type of bond in (1) is labile in the presence of the dehydronated amino group, resulting in the formation of (2). Such migrations of platinum are quite uncommon and this is the first case in which a Pt migrates from a sulfur to a nitrogen and *vice versa*.

In all Pt complexes described in this section, coordination to the sulfur atom is observed (i.e., with SAH, cysteine, methionine, GSH, MT, and other proteins), which is consistent with a high reactivity of sulfur. It is therefore reasonable to expect that significant amounts of platinum antitumor drugs will bind *in vivo* to sulfur-containing biomolecules. Although the kinetic reactivity of sulfur is high, the Pt—S bond of the methionine type is labile in the presence of other nucleophiles (*vide supra* for reactions with Naddtc and the interconversion of the Pt–SAH adducts). The relatively labile Pt—S methionine bond, but not the Pt—S cysteine bond, is likely to be relevant for the following cases:

1. The mechanism of suppressing the nephrotoxicity, i.e., Naddtc

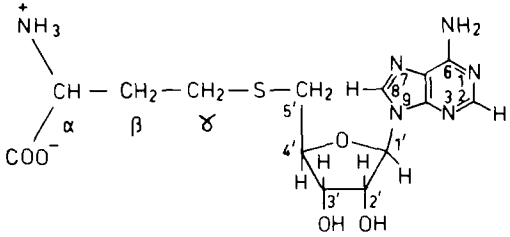


FIG. 15. Structure of *S*-adenosyl-L-homocysteine.

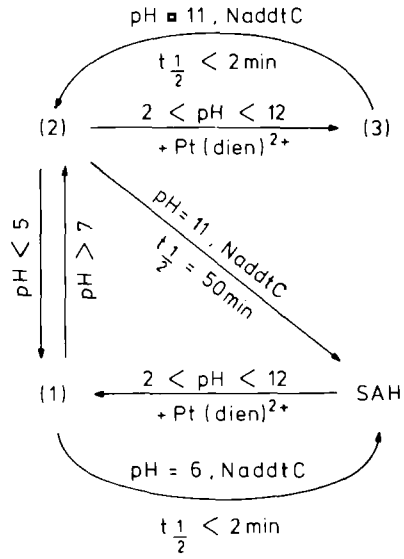


FIG. 16. Formation scheme of the complexes of SAH and their interconversions as a function of pH as well as decomposition reactions with Naddtc. Values for $t_{\frac{1}{2}}$ are also indicated.

reduces the nephrotoxicity by reversing Pt—S bonds only of the methionine type.

2. The development of permanent nephrotoxicity. Nephrotoxicity cannot be cured by Naddtc and thiourea when administered more than 4 hours after *cis*-Pt treatment. A possible mechanism for the development of permanent nephrotoxicity might be that the platinum migrates from a methionine to a cysteine within a protein, resulting in irreversible binding.

3. Acting as a drug reservoir for platination at DNA. Although there is no evidence for the existence of such a phenomenon, in principle Pt–methionine interactions are labile and therefore are likely candidates to serve as a drug reservoir for platination at DNA, i.e., spontaneous release and binding to DNA or nucleophilic displacement of platinum by guanine, for example.

V. Prospects for Future Studies on Pt Antitumor Compounds

Despite many intensive efforts to elucidate the detailed working mechanism of *cis*-Pt, as yet there is no conclusive proposal. It has been shown that both the cis and the transisomer can bind to DNA *in vivo*.

The difference in antitumor properties is thought to originate from differences in platinum–DNA adduct induction. As intrastrand chelation of neighboring purines is possible with *cis*-Pt, but—for steric reasons—not for *trans*-Pt, the AG and the GG chelates are likely candidates for the crucial lesion. A reasonable hypothesis states that these lesions, contrary to the GNG chelate and the interstrand cross-links, which can be formed by *trans*-Pt, also, are less or not at all recognized by cellular repair mechanisms, but on the other hand do interfere with DNA replication. Also, other important questions concerning the uptake of *cis*-Pt into cells (are certain cell walls selective for uptake of *cis*-Pt?) and the cellular transport (i.e., resulting in toxic side effects) and degradation are far from being answered. These questions promise a very interesting future for interdisciplinary research by chemists, biologists, pharmacologists, and physicians.

It is quite likely that the molecular basis of all kinds of toxicity, of resistance, and of repair will gain increasing interests in the coming years, and the first results are becoming available. Very recently, a factor in mammalian cells has been identified by Lippard, Donahue, and Chang *et al.* (*194–196*) that binds to *cis*-Pt-damaged DNA, but *not* to DNA modified with *trans*-Pt. This so-called DRP (damage recognition protein) could act in one of the following possibilities (*72*):

1. It could be a damage-repair protein, which would not work so well in (certain) tumor cells.
2. It could be a tumor-regulating protein, i.e., specific for tumor cells, and Pt-binding could perhaps deregulate the protein.
3. It could be a protein that would prevent Pt-damage repair by (other) proteins.

For an overview in the area of resistance, the reader is referred to recent contributions (*197–199*) dealing with suggestions to circumvent resistance.

Nephrotoxicity has also been reviewed recently (*200–202*), whereas Borch and Markman (*203*) have published an interesting paper on the modulation of *cis*-Pt toxicity, by using a combination of Naddtc and hypertonic saline, for example.

Finally, considerable progress has been reported about the possible use of Pt(IV) compounds, as in Fig. 2c, but with carboxylates as axial ligands, as oral anti-tumor drugs. (*204*).

Very recently, it has been observed that *E. coli* produces UvrABC excision repair proteins, and that the UvrAB complex binds to the convex side of a cisplatin-induced kink in DNA (*205*) It would be of great interest to study the similarities between this complex and the DRP protein mentioned above.

The results discussed in Section IV have clearly shown that there is increasing evidence for the importance of Pt–sulfur interactions from a bio(chemical) and medical point of view. Although such interactions are not responsible for the antitumor activity of *cis*-Pt, they probably contribute to some of the mentioned overall negative effects (inactivation, resistance, and nephrotoxicity). Much more research is needed to study these negative effects in detail. This could ultimately lead to compounds with better antitumor properties and lower side effects.

Some prospects for future studies from a chemical point of view are as follows:

1. Detailed kinetic studies to determine the rate-limiting step in the binding of Pt to S. This will be important in understanding the mechanisms of inactivation and resistance in normal tissues and tumors.
2. Systematic binding studies of a variety of Pt compounds with sulfur-containing molecules, which would eventually lead to the development of new antitumor drugs with structural properties such that the ratio of Pt binding at DNA compared to that of RSR is increased.
3. Extensive exchange reactions of platinated sulfurs of the methionine type with mononucleotides and longer DNA fragments. This is with the aim to investigate the possibility that such Pt—S methionine bonds could act as drug reservoirs for platination at DNA.
4. Exchange reactions of rescue agents with platinated proteins, which are known to form Pt—S cysteine bonds, to test the hypothesis (*131*) that the Pt—S cysteine bond is inert; this is important in the unraveling of the mechanism of the nephrotoxicity.
5. Ongoing Pt-binding studies with S-containing biomolecules and derivatives with the purpose to investigate in detail Pt migration reactions and competitions between a sulfur and the reactive guanine N7.

Abbreviations

cis-Pt	[*cis*-$PtCl_2(NH_3)_2$]
trans-Pt	[*trans*-$PtCl_2(NH_3)_2$]
CBDCA	[*cis*-$Pt(C_6H_6O_4)(NH_3)_2$]
DNA	deoxyribonucleic acid
RNA	ribonucleic acid
i.v.	intravenously
i.p.	intraperitoneally
Am	amine

diam	didentate amine
en	ethylenediamine
dien	diethylenetriamine
dach	1,2-diaminocyclohexane
damch	1,1-bis(aminomethyl)cyclohexane
i-$PrNH_2$	*i*-propylamine
N-het	heterocyclic amine
mepy	methylpyridine
R'R"SO	substituted sulfoxide
Me_2SO	dimethyl sulfoxide
MeBzSO	methyl benzyl sulfoxide
GSH	γ-L-glutamyl-L-cysteinylglycine
GS^-	GSH, dehydronated at the thiol group
GS-Me	*S*-methyl glutathione
MT	metallothionein
cys	cysteine
met	methionine
SAH	*S*-adenosyl-L-homocysteine
Naddtc	sodium diethyldithiocarbamate
STS	sodium thiosulfate
WR-2721	[*S*-2-(3-aminopropylamino)ethyl phosphorothioic acid]
WR-1065	dephosphorylated WR-2721
mesna	sodium 2-mercaptoethane sulfonate
d	deoxyribo
G	guanine
Guo	guanosine
GMP	guanosine monophosphate
p	phosphate group (irrespective of charge)
r_b	number of Pt atoms bound per nucleobase
CSA	chemical shift anisotropy

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