Loss of Ammine from Platinum(II) Complexes: Implications for Cisplatin Inactivation, Storage, and Resistance**

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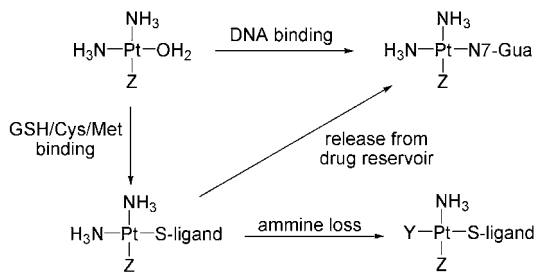
Abstract: Potential consequences of the binding of the anticancer drug cisplatin to various biomolecules in the cell have been investigated by using a combined density functional theory and continuum dielectric model approach. Since the ammine ligands remain coordinated at the metal upon formation of the most frequent DNA adducts, whereas they were found to be displaced from the metal upon formation of drug metabolites, we have analyzed the factors governing ammine loss from platinum(II) complexes as a possible pathway of cisplatin inactivation. The calculations systematically show the effect of 1) the *trans* ligand, 2) the charge of complex, 3) the nucleophile, and 4) the environment on the thermodynamic instability and kinetic lability of the platinum–ammine bonds. After initial binding of cisplatin hydrolysis products to thioethers or thiols, loss of the ammine *trans* to this sulfur ligand rather than replacement of the sulfur ligand itself by other nucleophiles like guanine-N7 is predicted to be the predominant reaction. The results of this study contribute to an understanding of the modes of cisplatin inactivation prior to DNA binding, for example, by elevated glutathione levels in cisplatin-resistant cancer cells.

Keywords: antitumor agents · cisplatin · density functional calculations · platinum

Introduction

Since the discovery of the anticancer activity of *cis*-diamminedichloroplatinum(II) (cisplatin),[1] tremendous research efforts have focused on the clarification of the mode of action of cisplatin and the development of metal-complex-based anticancer drugs.[2] After injection into the bloodstream, cisplatin reaches the cell by passive diffusion through the plasma membrane or by transport with the copper transporter Ctr1.[3] Due to the small intracellular chloride concentration (~10 mM) relative to that in extracellular fluids (~100 mM), one or both platinum–chloro bonds are hydrolyzed inside the cell. The activated hydrolyzed species may react with various biomolecules, in particular with adjacent guanine-N7 sites of genomic DNA to give 1,2-intrastrand cross-links. It was also suggested that the reaction of hydrolyzed cisplatin derivatives with sulfur ligands gives a storage form from which the diammineplatinum(II) moiety is slowly released to DNA (Scheme 1).[4] The kinked and partially unwound DNA at the platinated sites is recognized by proteins like those containing a high mobility group (HMG) domain.[5] Binding of these proteins to platinated DNA deprive them of their normal function, for example, of acting elsewhere as transcription factors (hijacking model), or shield the platinated sites from the binding of repair proteins, thus preventing DNA repair (shielding model). A complex cellular response to cisplatin treatment eventually leads to cell death (apoptosis or necrosis). Both modes of



Scheme 1. DNA binding, competitive sulfur-ligand binding, potential storage, and inactivation of cisplatin derivatives in the cell (simplified, charge of complexes not shown). Z = spectator ligand, Y = nucleophile.

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cell death are linked by the fact that certain caspases, that is, cysteine proteases involved in the execution phase of apoptosis, can cleave poly(ADP-ribose)polymerase, which catalyzes the polymerization and consequent depletion of oxidized nicotinamide adenine dinucleotide (NAD^+) in necrosis.[6]

A major drawback in the chemotherapy of certain cancers with cisplatin is the resistance to the drug. While cisplatin is particularly active in the treatment of testicular cancer, some cancer types, like colorectal cancer, do not respond to cisplatin (intrinsic resistance) and others like ovarian cancer initially do, but then become resistant during therapy (acquired resistance).[7] Several mechanisms of cisplatin resistance have been identified and can be classified into scenarios prior and subsequent to DNA binding. Pre-binding mechanisms include the reduced accumulation and the increased inactivation of the drug in the cell, while post-binding mechanisms include increased nucleotide excision repair (NER) of the DNA adducts as well as reduced mismatch repair (MMR). Cells with an intact MMR system may be more sensitive to the drug due to the induction of apoptosis by futile MMR cycles resulting from a continuous direction of the repair attempts to the non-platinated strand.[5a,6]

We believe that an important molecular event in the intracellular inactivation of cisplatin is the displacement of one or both ammine ligands from the metal, because the most frequent cisplatin–DNA adducts, diammine-1,2-intrastrand-GG cross-links, as well as several other cisplatin–DNA adducts still contain both ammine ligands.[5a] Diammine-1,2-intrastrand-GG cross-links cannot form from transplatin, the pharmacologically inactive geometric isomer of cisplatin. A reaction involving ammine loss from a metal complex is thermodynamically and kinetically more favorable than the corresponding reverse reaction due to protonation of the released ammonia (pK_a of ammonium: 9.25).[8] The function of the ammine and amine ligands of platinum anticancer complexes has been controversial. To be anticancer-active, "classic" platinum(II) complexes of the type $[PtL_2Cl_2]$ require at least one N–H bond in the ammine or amine ligand L, with the activity increasing in the order $L = NR_3$ (inactive; R = alkyl) $\ll NHR_2 < NH_2R < NH_3$ (most active).[9] N–H bonds may act as hydrogen-bond donors to the 5′-phosphate of d(pGpG) moieties, as suggested by molecular mechanics calculations[10] and an X-ray structure of a platinated duplex dodecamer.[11] The preference of cisplatin for guanine (G) over adenine (A) appears to be related to the basicity of G-N7 and to hydrogen bonding involving G-O^6 or repulsion from A-N^6H_2.[12] It has also been suggested that the very small size of the N–H group, not its hydrogen-bonding ability, is responsible for the good activity exhibited by Pt compounds with amine carrier ligands bearing multiple N–H groups.[13] Recent theoretical studies indicated that an (ammine)N–H···O^6(G) hydrogen bond in Pt–G adducts[14] is less important than that in the transition states of their formation.[15] A mechanism in which ammine loss may activate rather than inactivate the drug was also suggested, because cisplatin derivatives of the type *cis*-$[Pt(NH_3)_2Cl(C\text{-}N3)]^+$ (C = cytosine) undergo replacement of the ammine *trans* to the chloro ligand by another chloro ligand. This scenario may finally lead to cisplatin adducts containing three biomolecules.[16] Moreover, several "non-classic" platinum complexes were demonstrated to be anticancer-active as well, which is possibly attributed to other modes of action.[2]

There is strong experimental evidence that certain biomolecules like methionine residues or γ-glutamylcysteinylglycine (glutathione, GSH) can displace the ammine from cisplatin derivatives prior to DNA binding,[17] and elevated GSH levels were detected in cisplatin-resistant cell lines.[7d] Nevertheless, the questions when and why the ammine leaves have not been addressed satisfactorily, neither in vivo, in vitro, nor in silico. Whereas many recent quantum chemical in silico studies[14,15,18,19] in the platinum anticancer arena focused on cisplatin hydrolysis as well as binding to the purine bases of DNA, we present a quantum chemical study on reactions of cisplatin that may prevent the drug from reaching its ultimate DNA target. This work aims 1) to analyze the factors that determine the thermodynamic instability and kinetic lability of platinum–ammine bonds and 2) to help resolve the storage controversy, that is, clarify whether the reaction of cisplatin derivatives with intracellular sulfur ligands inactivates the drug due to subsequent ammine displacement or yields a drug reservoir from which the platinum complex is slowly released to DNA (Scheme 1).

Results and Discussion

Factors governing ammine loss from platinum(II) complexes: The methods employed for this work, density functional theory (DFT) at the B3LYP level together with a continuum dielectric model (CDM) at a dielectric constant ε of 80 representing water, are described in reference [19]. Former DFT studies addressed more straightforward questions, for example, how guanine, adenine, and methionine compete for the reaction with cisplatin derivatives.[18,19] In contrast, the present work deals with a large amount of nucleophilic substitution reactions of cisplatin that are potentially relevant to drug inactivation, storage, and resistance. In order to investigate the general trends in the reaction and activation free energies for ammine displacement, we decided in favor of the following strategy: We consider $[Pt(NH_3)_4]^{2+}$ as the reference complex and NH_3 as the reference nucleophile, and explore systematically the effect of 1) the *trans* director T, 2) the charge of complex, 3) the nucleophile Y, and 4) the environment on the thermodynamics and kinetics of the nucleophilic substitution reactions (Figure 1). Various functional groups of biomolecules (illustrated here) have been considered.[20] The results are summarized in the following four sections.

The trans director: To investigate the thermodynamic *trans* influence,[21] which is defined as the extent to which a ligand T of a metal complex weakens the bond *trans* to itself, the

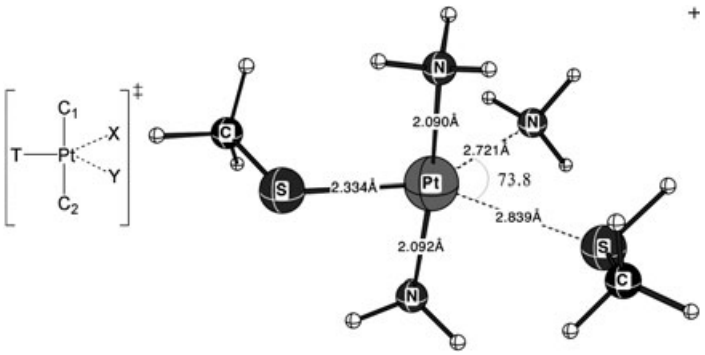
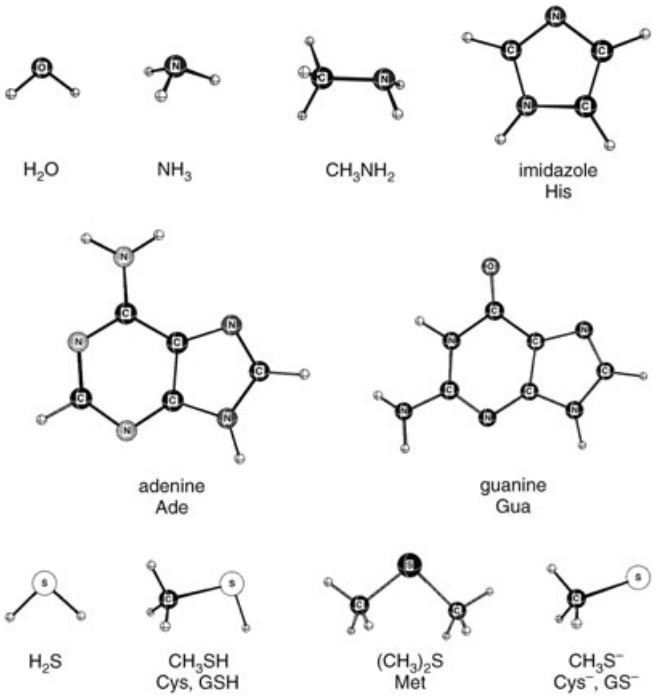


Figure 1. Transition structure for the nucleophilic substitution at Pt^{II} centers. Left: Schematic representation with Y = nucleophile, X = leaving group, T = *trans* ligand, C_1, C_2 = *cis* ligands. Right: Calculated structure with Y = MeSH, X = NH_3, T = MeS^-, and C_1, C_2 = NH_3. Distances in Å, angles in degrees.



bond dissociation free energies (BDFEs) for the Pt–NH_3 bond *trans* to T in $[Pt(NH_3)_3T]^{2+/+}$ complexes have been calculated at $\varepsilon = 80$. The results are displayed in Figure 2 as large empty squares. The calculations show the Pt–NH_3 BDFEs for various *trans* ligands T decreasing in the following order: aqua (~25 kcal mol^{-1}) ≫ nitrogen heterocycles like Gua, Ade, His (~19 kcal mol^{-1}) > ammine (~16 kcal mol^{-1}) ~ neutral sulfur ligands like Cys and Met (~16 kcal mol^{-1}) ≫ the anionic sulfur ligand Cys^- (~10 kcal mol^{-1}). To investigate the kinetic *trans* effect,[21] which is defined as the effect of a ligand T on the rate of substitution of the group *trans* to T, the activation free energies (ΔG_a) for the substitution of the ammine group *trans* to T in $[Pt(NH_3)_3T]^{2+/+}$ complexes by nucleophilic attack of another ammine group have been predicted. The ΔG_a are displayed in Figure 2 as large filled

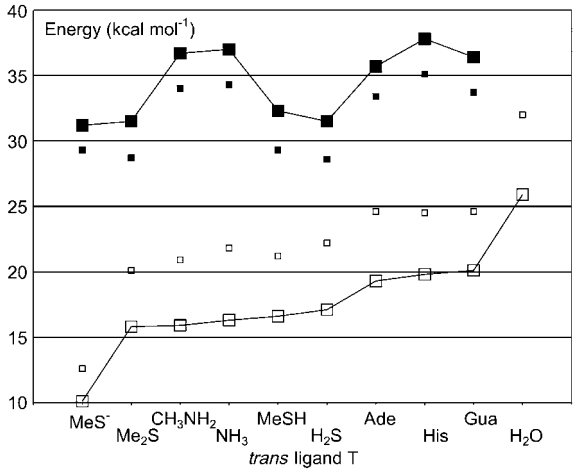


Figure 2. BDFE's (in kcal mol^{-1}) for the dissociation of the Pt–NH_3 bond *trans* to T in $[Pt(NH_3)_3T]^{2+/+}$ complexes (*trans* influence, empty symbols) and activation free energies ΔG_a (in kcal mol^{-1}) for the exchange of the NH_3 *trans* to T by another NH_3 (*trans* effect, filled symbols) calculated at $\varepsilon = 80$ (large symbols) and $\varepsilon = 9$ (small symbols).

squares and indicate that the kinetic *trans* effect of the biomolecules considered significantly differs from the thermodynamic trans influence, identifying three classes of ligands: 1) No *trans* effect for T = aqua, because all the reaction paths obtained lead to the displacement of the aqua ligand rather than to ammine displacement. 2) A weak *trans* effect (ΔG_a ~ 36–38 kcal mol^{-1}) for T = all nitrogen ligands. 3) A stronger *trans* effect (ΔG_a ~ 31–32 kcal mol^{-1}) for T = all neutral (Cys, Met) and anionic (Cys^-) sulfur ligands.[22] The following sections will focus on metabolites of the drug that contain sulfur ligands *trans* to the leaving ammine, because the fate of cisplatin in the cell is kinetically controlled and thiolates, thiols, and thioethers show the largest trans effect.

The charge of the complex: To investigate the influence of the charge of the complex on the thermodynamic instability of the platinum–ammine bonds, we have predicted the Pt–NH_3 BDFE's in the *cis*-diammine thiol/thiolate complexes, *cis*-$[Pt(NH_3)_2(MeSH)_n(MeS)_{2-n}]^{n+}$ ($n = 0, 1, 2$). The results given in Figure 3 reveal that the BDFE's are significantly lower only if the *trans* ligand is the anionic thiolate Cys^- (BDFE = 7–11 kcal mol^{-1}). The sulfur ligands *cis* to the ammine and the charge of the complex have a relatively minor effect on the BDFE's. The calculated ΔG_a for the substitution of the ammines in *cis*-$[Pt(NH_3)_2(MeSH)_n(MeS)_{2-n}]^{n+}$ ($n = 0, 1, 2$) upon nucleophilic attack of another ammine are also given in Figure 3. In contrast to the thermodynamics, the kinetics is independent of whether the *trans* ligand T is thiol or thiolate, as indicated by ΔG_a of 30–32 kcal mol^{-1}. The calculated pK_a values (Figure 3) demonstrate metal binding to lower the pK_a of thiols in the dicationic complexes (calcd pK_a ~ 3) relative to free MeSH (calcd pK_a ~ 9), implying that dicationic thiol complexes become deprotonated easily. In contrast, monocationic thiol complexes (calcd pK_a ~ 7) become deprotonated less easily,

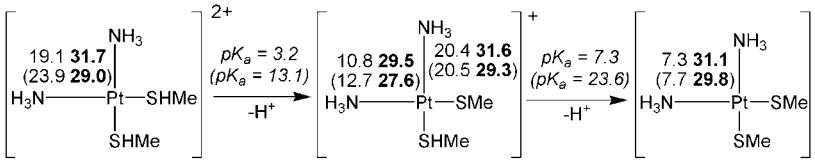


Figure 3. BDFE's (in kcal mol^{-1}) for the dissociation of the Pt–NH_3 bonds of thiol and thiolate complexes (plain text), activation free energies ΔG_a (in kcal mol^{-1}) for the exchange of the NH_3 by another NH_3 (bold), and predicted pK_a's at $\varepsilon = 80$ (italics). Values at $\varepsilon = 9$ are given in parentheses.

so both the monocationic and neutral forms may exist at physiological pH. However, the protonation state has little consequence for the kinetic *trans* effect.

The nucleophile: To clarify which biomolecules may displace the ammine most easily from the metal, we have predicted the reaction free energies (ΔG_r, Figure 4, large empty circles) and activation free energies (ΔG_a, Figure 4, large filled

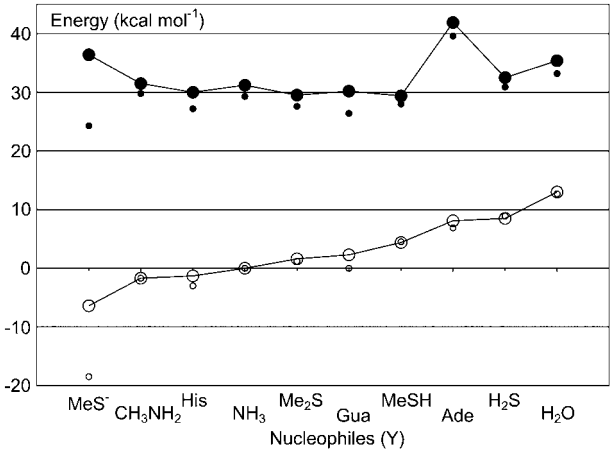


Figure 4. Reaction free energies ΔG_r and activation free energies ΔG_a (in kcal mol^{-1}) for the substitution of NH_3 *trans* to MeS^- in $[Pt(NH_3)_3(MeS)]^+$ complexes by various nucleophiles. Values calculated at $\varepsilon = 80$ (large symbols) and $\varepsilon = 9$ (small symbols).

circles) for the nucleophilic substitution of the ammine *trans* to methanethiolate in *cis*-$[Pt(NH_3)_3(MeS)]^+$ by various nucleophiles Y. Based on the results of the former sections, methanethiolate (Cys^-) has been chosen as the *trans* director. The calculations show the ΔG_r to decrease in the following order: Y = water (13) > Ade (8) > Cys (4) > Met, Gua, His (−2 to +2) > Cys^- (−6 kcal mol^{-1}). Whereas the reaction of the thiolate is thermodynamically most favorable, this species is kinetically one of the weakest nucleophiles ($\Delta G_a = 36$ kcal mol^{-1}). Various other nucleophiles have significantly smaller activation barriers ($\Delta G_a = 29$–32 kcal mol^{-1}), including the neutral sulfur and nitrogen nucleophiles Met, Cys, and Gua. Hence, a variety of N and S nucleophiles may displace the ammine, but Ade and thiolates can be excluded. Thiols may react with the metal complex and become deprotonated subsequently, forming a very stable platinum–thiolate bond.

The environment: To investigate the trend in the reaction and activation free energies in a less polarizable environment provided by proteins, we compare the reaction and activation free energies at $\varepsilon = 80$ and 9. The latter ε gives an estimate of the ΔG_r and ΔG_a at solvent-exposed sites of proteins.[23] Figures 2 and 4 display the results at $\varepsilon = 9$ as small symbols; Figure 3 displays the values at $\varepsilon = 9$ in parentheses. Although a lower ε weakens the *trans* influence (i.e., increases the Pt–NH_3 BDFEs, see Figure 2), it increases the *trans* effect (i.e., decreases the ΔG_a for ammine substitution trans to T), indicating that the kinetically controlled ammine displacement can be promoted by a protein environment. The pK_a of the thiol complexes (Figure 3) at $\varepsilon = 9$ are predicted to be significantly higher than those at $\varepsilon = 80$, indicating that metal-bound thiols do not become deprotonated in a low dielectric medium. The effect of the dielectric medium on the ΔG_r and ΔG_a for the nucleophilic substitution of the ammine *trans* to methanethiolate (Figure 4) considerably depends on the nucleophile. The activation barrier with guanine as the nucleophile is lowered by as much as 4 kcal mol^{-1} upon decrease of ε from 80 to 9, which is likely attributed to the polarizability of the aromatic heterocycle.

Cisplatin storage versus inactivation: To assess the storage hypothesis,[4] we have investigated the substitution of Me_2S and MeS^- in *cis*-$[Pt(NH_3)_3(Me_2S)]^{2+}$ and *cis*-$[Pt(NH_3)_3(MeS)]^+$, respectively, by guanine. Adducts of the types *cis*-$[Pt(NH_3)_2Z(thioether)]^{2+/+}$ and *cis*-$[Pt(NH_3)Z(thiolate)]^{+/0}$ may be formed in the cell,[24] because it was previously shown that the binding of thioethers or thiols to platinum(II) complexes in water is kinetically competitive if not superior to the binding of the purine bases.[4,19,25] The adducts of sulfur ligands may serve as a "drug reservoir", from which the platinum–diammine is slowly released to its DNA target. Alternatively, the ammine rather than the sulfur ligand may be displaced by biomolecules in the cell (Scheme 1). Note that several former experimental competition studies that considered 1,2-diaminoethane (en) or 1,5-diamino-3-azapentane (dien) complexes of platinum(II) did not solve the storage controversy, because the Pt–en and Pt–dien bonds are more stable and more inert than are platinum(II)–ammine bonds due to the increase of translational and rotational entropy upon release of the monodentate ammine ligands (chelate effect).[25] Our calculations reveal very high activation free energies at $\varepsilon = 80$ for the substitution of Me_2S (40 kcal mol^{-1}) and MeS^- (44 kcal mol^{-1}) in *cis*-$[Pt(NH_3)_3(Me_2S)]^{2+}$ and *cis*-$[Pt(NH_3)_3(MeS)]^+$, respectively, by Gua. Recall that the ΔG_a values for the replacement of the ammine group *trans* to the sulfur ligand in *cis*-$[Pt(NH_3)_3$-

$(Me_2S)]^{2+}$ and *cis*-$[Pt(NH_3)_3(MeS)]^+$ are considerably lower, approximately 31–32 kcal mol^{-1} at $\varepsilon = 80$. At $\varepsilon = 9$, the ΔG_a for the substitution of Me_2S in *cis*-$[Pt(NH_3)_3(Me_2S)]^{2+}$ decreases to 34 kcal mol^{-1}, but the *trans* effect of the sulfur ligands leading to ammine loss is also enhanced by a less polarizable environment (vide supra). Hence, theory does not support the transient platination of sulfur ligands and subsequent transfer of the diammineplatinum core to genomic DNA as a predominant mechanism in the mode of action of the drug. However, the calculations do allow the possibility of the formation of protein–DNA cross-links through binding of a thioether or thiol functional group of a protein and subsequent replacement of the ammine *trans* to this sulfur ligand by another nucleophile, such as a guanine-N7 site. This scenario may lead to the formation of cisplatin adducts containing three biomolecules.

Conclusions

In summary, we have explored by a systematic DFT/CDM approach ($\varepsilon = 80$) the *trans* influence and *trans* effect of various biomolecules in platinum(II) complexes and their reactions that are potentially relevant to cisplatin inactivation upon ammine loss. Whereas thiolates (Cys^-) show the largest thermodynamic *trans* influence, anionic and neutral sulfur ligands (Cys^-, Cys, Met) show the largest kinetic *trans* effect. The charge of a Pt^{II} complex has a relatively minor effect on the activation free energies for ammine displacement. Several nitrogen and sulfur nucleophiles like Gua, Cys, and Met are able to replace the ammine *trans* to a sulfur ligand, whereas the Pt–NH_3 bonds are inert to nucleophilic attack by Ade and Cys^-. A less polarizable environment ($\varepsilon = 9$), which is likely present when amino acid residues of proteins react with the drug, reduces the *trans* influence, enhances the *trans* effect, and inhibits deprotonation of metal-bound thiols. Theory does not support the platination of sulfur ligands and subsequent metal release to DNA ("storage hypothesis") as a key mechanism in the mode of action of cisplatin, because the activation free energies for the replacement of Cys^- or Met by Gua in cisplatin derivatives containing an intact *cis*-diammine core are considerably higher than the activation free energies for the substitution of the ammine group *trans* to an anionic or neutral sulfur ligand by Gua. Additional studies are required to understand entirely how platinum drug resistance can be overcome by "third-generation" anticancer complexes,[2] which contain nitrogen ligands other than ammine.

Computational Methods

Structures and energies: The geometries of molecules and transition states (TS) were optimized at the gradient-corrected DFT level using the three-parameter fit of exchange and correlation functionals of Becke (B3LYP),[26] which includes the correlation functionals Lee, Yang, and Parr (LYP),[27] as implemented in Gaussian 98.[28] The LANL2DZ ECP's[29] and valence basis sets were used for platinum, and 6-31G(d,p) basis sets were used for the other atoms.[30] This basis-set combination is denoted II–. Vibrational frequencies were also calculated at B3LYP/II–. The structures reported are either minima (NIMAG = 0) or transition states (NIMAG = 1) on the potential-energy surfaces. Improved total energies were calculated at the B3LYP level by using the same ECP and valence basis set for the metals, but totally uncontracted and augmented with Frenking's set of f functions,[31] together with the 6-311+G(3d) basis sets for the sulfur and chlorine and the 6-311+G(d,p) basis sets for the other atoms. This basis-set combination is denoted III+ and was successfully employed for other reactions of third-row transition metals.[32] Activation and reaction free energies (ΔG_a, ΔG_r) were calculated by adding corrections from unscaled zero-point energy (ZPE), thermal energy, work, and entropy evaluated at the B3LYP/II– level at 298.15 K, 1 atm to the activation and reaction energies (ΔE_a, ΔE_r), which were calculated at the B3LYP/III+//II– level.

Solvation free energies: Solvation free energies $G_{solv}{}^{\varepsilon}$ of the structures optimized at the B3LYP/II– level (vide supra) were calculated by Poisson–Boltzmann (PB) calculations with a dielectric constant ε of the dielectric continuum that represents the solvent. The PB calculations were performed at the B3LYP level using the LACV3P++** basis set for platinum and the 6-31G** basis set for the other atoms as implemented in the Jaguar 5 program package.[33,34] The continuum boundary in the PB calculations was defined by a solvent-accessible molecular surface with a set of atomic radii for H (1.150), C (1.900), N (1.600), O (1.600), S (1.900), Cl (1.974), and Pt (1.377 Å).[35] The pK_a predictions were carried out by using a thermodynamic cycle [Eq. (1)][36] in which ΔG^1 and ΔG^{ε} are the reaction free energies of the reaction, $AH \rightarrow A^- + H^+$, in vacuo and at ε, respectively, $G^{\varepsilon}_{solv}(X)$ is the solvation free energy of species AH or A^- at ε obtained through the PB calculations, R is the ideal gas constant, and T is the temperature (298.15 K).

$$\Delta G^{\varepsilon} = \Delta G^1 + G^{\varepsilon}_{solv}(H^+) + G_{solv}{}^{\varepsilon}(A^-) - G^{\varepsilon}_{solv}(A)$$
$$pK_a{}^{\varepsilon} = \Delta G^{\varepsilon}/RT \ln 10 \qquad (1)$$

The experimental value of −260.9 kcal mol^{-1} was used for the hydration free energy of the H^+ ion ($G^{80}_{solv}(H^+)$),[37] as recommended by Truhlar and co-workers.[37a] The solvation free energies G^{ε}_{solv} of the H^+ ion at various ε were calculated using a scaling equation derived from the Born model.[38] A more detailed description of the methods was presented in reference [19].

The present computational approach is clearly superior to recent studies in vacuo. Some of its limitations, however, are demonstrated by the prediction of a slightly larger activation free energy for the reaction of the first cisplatin hydrolysis product, *cis*-$[Pt(NH_3)_2(OH_2)Cl]^+$, with Me_2S (~27 kcal mol^{-1}) compared to that with Gua (~25 kcal mol^{-1}),[19] while the reaction of cisplatin with the Met moiety in a peptide–oligonucleotide hybrid yielding *cis*-$[Pt(NH_3)_2(Met\text{-}S)(Cl/OH_2)]^{+/2+}$ was experimentally observed to be kinetically preferred to that with the Gua moiety of the hybrid.[17d] A second example is the lability of an ammine *trans* to a chloro ligand in cisplatin derivatives of the type *cis*-$[Pt(NH_3)_2Cl(C\text{-}N3)]^+$ (C = cytosine) with respect to replacement by another chloride, which was experimentally observed,[16a] while the calculations at B3LYP together with the PB approach do not predict significantly different activation free energies for the replacement of the ammine *trans* to the chloro ligand in *cis*-$[Pt(NH_3)_2Cl(C\text{-}N3)]^+$ and $[Pt(NH_3)_3Cl]^+$ by another chloro ligand (38.2 vs. 36.2 kcal mol^{-1}). Further investigation of these last reactions by using the second-order Møller–Plesset perturbation theory (MP2) ab initio method led to activation free energies similar to those at B3LYP (Table S6 in the Supporting Information). Consideration of an alternative definition of the molecular cavity based on atom types (like carbon-sp^2), which we in general do not prefer due to its arbitrariness in studies of reactions involving changes of atom types, did not change the results significantly in comparison with the present atom-based definition of the molecular cavity. Although it is typically claimed that continuum dielectric models include entropic corrections to solvation free energies in the parameters defining the molecular cavity, activation free energies of bimolecular reactions (like the substitution reactions described in this work) may contain systematic errors, that is, the ΔG_a of *all* such reactions

are predicted to be too large by few kcal mol^{-1}. This drawback might be overcome by scaling the calculated gas-phase entropies, because Wertz showed that the molecules of water, ammonia, *n*-octane, and *n*-octanol loose a constant fraction (~0.46) of their entropies when they are dissolved in water.[39] Hence, the interpretation of the calculated results should focus on trends rather than on a particular number. Reactive Car–Parrinello molecular dynamics approaches that consider the solvent explicitly might improve the results in future work.

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