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Studies of interactions between platinum(II) complexes and some biologically relevant molecules

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Abstract—The reactions of Pt(II) complexes, *cis*-$[Pt(NH_3)_2Cl_2]$, $[Pt(terpy)Cl]^+$, $[Pt(terpy)(S\text{-cys})]^{2+}$, and $[Pt(terpy)(N7\text{-guo})]^{2+}$, where terpy = 2,2′:6′,2″-terpyridine, *S*-cys = L-cysteine, and N7-guo = guanosine, with some biologically relevant ligands such as guanosine-5′-monophosphate (5′-GMP), L-cysteine, glutathione (GSH) and some strong sulfur-containing nucleophiles such as diethyldithiocarbamate (dedtc), thiosulfate (sts), and thiourea (tu), were studied in aqueous 0.1 M Hepes at pH of 7.4 using UV–vis, stopped-flow spectrophotometry, and ^1H NMR spectroscopy.

1. Introduction

Cisplatin, *cis*-$[Pt(NH_3)_2Cl_2]$, and carboplatin, $[Pt(NH_3)_2(O,O\text{-cbdca})]$, where cbdca is cyclobutane-1,1-dicarboxylate, are widely used anticancer drugs for the treatment of testicular, ovarian, head, neck, and non-small cell lung cancer. Although there is some evidence to suggest that other biological targets are important in the mechanism of cisplatin, it is generally accepted that the anti-tumor activity of platinum drugs can be ascribed to interactions between the complex and DNA.[1–7] However, there are many other potential biomolecules that can also react with these Pt(II) complexes, such as small molecules, proteins, and enzymes. In fact, already in the blood, where the Pt drug is administered by injection or infusion, several molecules are available for kinetic and thermodynamic competition.[3] The binding to DNA eventually leads to an altered protein conformation and changes in biological activity, especially when enzymatic reactions are affected. Sulfur-containing molecules have a high affinity for platinum and could form very stable bonds. Moreover, the interaction of Pt complexes with sulfur-containing biomolecules has been associated with negative phenomena, such as nephrotoxicity, gastrointestinal toxicity, ototoxicity, and neurotoxicity.[7–9] Cardiotoxicity also occurs during therapy with platinum drugs. Several cases of acute myocardial infarction after cisplatin therapy were reported. Cardiotoxicity includes a wide range of cardiac effects from small changes in blood pressure and arrhythmias to cardiomyopathy. Several factors have been suggested to be involved like vascular damage, alterations in platelet aggregation, and hypomagnesemia.[10–13]

Reactions with thiol (SH) groups of protein side chains (e.g., in metallothionine and glutathione) are thought to trap and deactivate the drug before it reaches its cellular DNA target to form 1,2-intrastrand cross-links with guanine bases, the likely cytotoxic adduct.[14] At present it is not clear how the platinum(II) species reach the DNA, because Pt(II) has high affinity for binding to sulfur donors, especially thiols, compared to nitrogen donor ligands such as DNA bases.[4,7] However, the intra-cellular concentrations of sulfhydryl groups including cysteine and glutathione could be as high as 10 mM. A conventional hypothesis is that sulfur-containing nucleophiles initially bind to the platinum atom and then convert to platinum–DNA complexes, thermodynamically more stable products. Pt–sulfur (thioethers) adducts have been postulated to be a drug reservoir for platinum at DNA and may act as intermediates of platinum compounds and transform them into Pt–DNA adducts.[15–20] At the same time, platinum drugs

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could be inactivated in the reactions with cysteine-rich proteins, like glutathione (GSH), or metallothionine (MT).[20] Moreover, these proteins could substitute coordinated bases of DNA from Pt–DNA adducts, and also thioethers from Pt-thioether products[17,18] forming very stable Pt-thiolate bonds. The intermolecular substitution of *S*-methylglutathione GSMe by GSH is much faster than the substitution by N7-guanine.[21] In this way the activity of Pt drugs could be much lower and their toxicity much higher. Nephrotoxicity has perhaps been the major problem in cisplatin treatment and it can be reduced including pre- and post-hydration and mannitol-induced diuresis. Therefore, several sulfur containing compounds, such as diethyldithiocarbamate (ddtc), sodium thiosulfate (sts), thiourea (tu), and glutathione (GSH), considered as so-called rescue agents or protective agents have been developed for use in co-administration with the aim to modulate the above side effects of platinum therapy (see Scheme 1).

The mono-functional $[Pt(terpy)Cl]^+$ and related complexes of the general type $[Pt(terpy)X]^{2+}$ (terpy = 2,2′:6′,2″-terpyridine) are very useful models for studying the ligand substitution reactions of square-planar complexes. We have reported the kinetics of the complex-formation reactions between $[Pd(terpy)H_2O]^{2+}$ and $[Pt(terpy)H_2O]^{2+}$ with L-cysteine, DL-penicillamine, glutathione, and thiourea.[22,23] We have also recently published the results for the complex formation of $[Pt(terpy)X]^{2+}$ with some nitrogen- and sulfur-bonding bio-molecules.[24,25]

With the aim to extend our earlier work,[22–25] we investigated and reported here study of the complex-formation kinetics of *cis*-$[Pt(NH_3)_2Cl_2]$, $[Pt(terpy)Cl]^+$, $[Pt(terpy)(S\text{-cys})]^{2+}$, and $[Pt(terpy)(N7\text{-guo})]^{2+}$ with some biologically important molecules such as: guanosine-5′-monophosphate (5′-GMP), glutathione (GSH), L-cysteine (*S*-cys) and with some nucleophiles which have been used as 'rescue agents' or 'protective agents': diethyldithiocarbamate (dedtc), sodium thiosulfate (sts), and thiourea (tu). The reactions were studied in aqueous solutions in 0.1 M Hepes as a buffer, and at physiological pH of 7.4, using UV–vis spectrophotometry, stopped-flow, and ^1H NMR techniques, Eqs. 1 and 4.

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

$L = 5'\text{-GMP and GSH}$

$$[Pt(terpy)Cl]^+ + L \rightleftharpoons [Pt(terpy)L]^{z+} + Cl^- \quad (2)$$

$L = GSH, S\text{-cys}, 5'\text{-GMP}, \text{tu}$

$$[Pt(terpy)(S\text{-cys})]^{2+} + L \rightleftharpoons [Pt(terpy)L]^{z+} + \text{L-cysteine} \quad (3)$$

$L = \text{tu, dedtc, sts}$

$$[Pt(terpy)(N7\text{-guo})]^{2+} + L \rightleftharpoons [Pt(terpy)L]^{z+} + \text{guanosine} \quad (4)$$

$L = \text{L-cysteine, GSH, tu, sts, dedtc}$

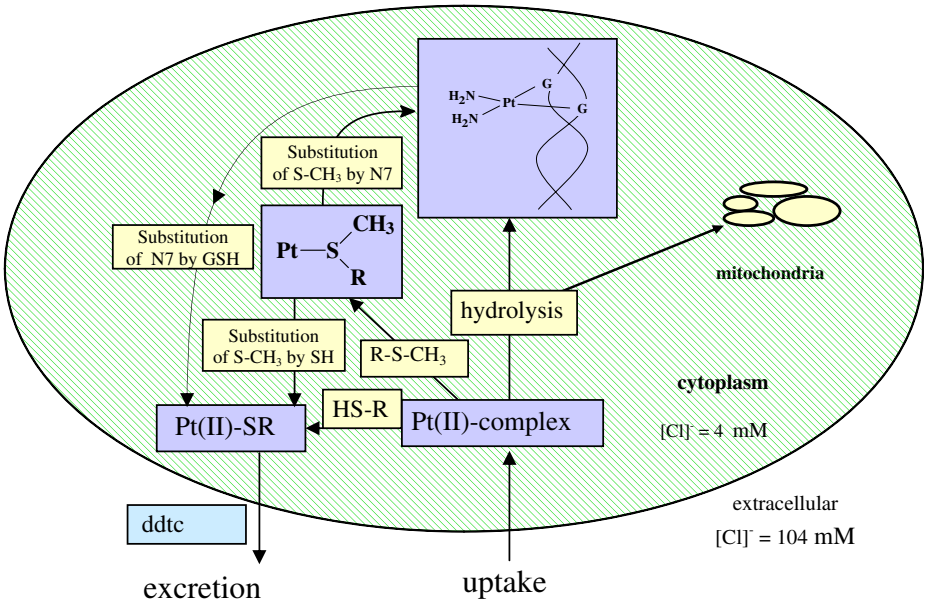
This set of nucleophiles was selected because of their difference in nucleophilicity, steric hindrance, binding properties, and biological relevance (structures are shown in Fig. 1).

It was envisaged that this study could throw more light on the mechanism of the interactions of platinum anti-tumor complexes with nitrogen- and sulfur-bonding ligands, and also provide a better understanding of the mechanism of chemo-protection and platinum metabolism.

2. Results and discussion

2.1. Reactions of cisplatin, *cis*-$[Pt(NH_3)_2Cl_2]$, with 5′-GMP and GSH

Mononuclear Pt(II) complexes of the type $[PtA_2X_2]$ (X = anionic leaving group; A = ammonia, amine, pyri-



Scheme 1.

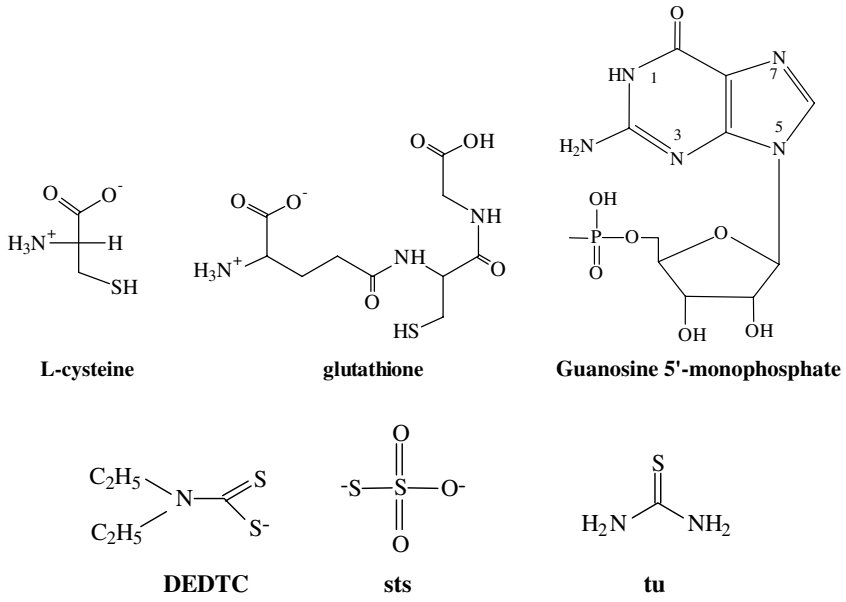


Figure 1. Structures of the nucleophiles.

dine), for example, cisplatin, *cis*-$[Pt(NH_3)_2Cl_2]$, and carboplatin, $[Pt(NH_3)_2(O,O\text{-cbdca})]$, are widely applied in medicine as anticancer agents and there is much interest in investigating and comparing the properties of such complexes under physiological conditions. Therefore, the reactions of cisplatin with 5′-GMP and GSH were studied at a pH of 7.4 at 37 °C in Hepes buffer. This buffer was selected because it is sterically more crowded than for instance Tris buffer (tris(hydroxymethyl)aminomethane) and does therefore not coordinate as efficiently to Pt(II) as Tris.[26] Both buffers are used in cell tests and DNA binding studies of Pt(II) drugs.

The reactions of cisplatin with 5′-GMP and GSH were studied spectrophotometricaly at 37 °C. NMR technique was also applied to study the reactions of cisplatin with guanosine-5′-monophosphate. Second-order rate constants, k_2, were obtained from Eq. 5.

$$k_2 t = x/a_0(a_0 - x) \quad (5)$$

where x is the amount of the product, $[Pt(NH_3)_2Cl(N7\text{-GMP})]^+$, and a_0 is initial concentration of cisplatin, *cis*-$[Pt(NH_3)_2Cl_2]$. Calculations were performed by relative integration (estimated error is 5%) of suitable proton signals of both reaction product and starting materials during the reaction (Table S1, Supplementary material). The values of the rate constant were determined from Guggenheim plot,[27] in which $x/a_0(a_0 - x)$ is plotted against time, showing a straight line passing through the origin as shown in Figure 2. The values of the second-order rate constants, k_2, were calculated from the slope of this line and are presented in Table 1 as well.

Figure 3 shows time course of the reaction between cisplatin and 5′-GMP. The peak for the free 5′-GMP is at δ 8.22 ppm, and for the product the peaks are at δ 8.69 and at 8.71 ppm. The peak at 8.69 ppm is smaller than the peak at 8.71 ppm and at the later stage of the reaction it disappears. However, during the reaction the peak at 8.71 ppm, which corresponds to the product, $[Pt(NH_3)_2Cl(N7\text{-GMP})]^+$, increased in intensity, while the peak for the free 5′-GMP (δ 8.22 ppm) decreased in intensity. At the end of the reaction all 5′-GMP is coordinated to Pt(II), and the peak for the free 5′-GMP disappears as shown in Figure 3.

The rate constants for the reactions of cisplatin with 5′-GMP, obtained by ^{1}H NMR experiments and obtained by UV–vis experiments, are in a good agreement. However, these results are in a good agreement with the previously published results.[16]

The reactions of cisplatin with GSH were studied spectrophotometricaly, and it has been found that GSH is better nucleophile for cisplatin than 5′-GMP, what is also in agreement with previously published results.[28,29] However, cisplatin readily reacts with glutathione and as much as 67% of the administered platinum has been found to coordinate to glutathione. Glutathione has been used as protecting agent and administered before or after cisplatin.[7] The role of glutathione appears to be dual: glutathione both deactivates and activates cisplatin.[30] The higher effectiveness of cisplatin has also been demonstrated by co-administering cisplatin and glutathione in patients. However, it is not clear whether this increase in effectiveness is due to the reduced toxicity or due to the modification of the platinum drug by binding to the metal. The platinum–glutathione adduct (Pt–GSH) is transported out of the cells after 12 h through an ATP-dependent transport mechanism. Currently, there is much interest in the mechanisms responsible for the development of resistance. Such resistance is often associated with increased cellular glutathione consistent with the view that glutathione protects cells against foreign compounds and the effects of radiation.[31]

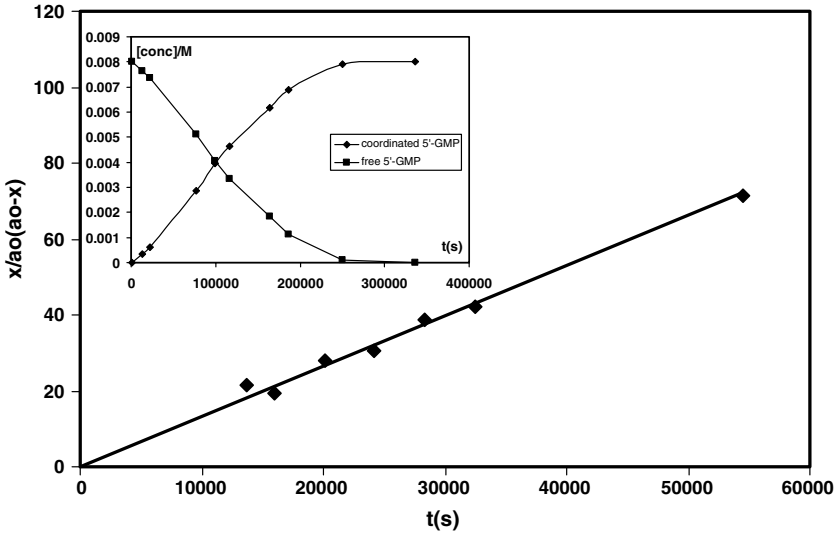


Figure 2. Second-order plot for the reaction of cisplatin (7.5 mM) with 5′-GMP (7.5 mM). The *y* axis represents the right-hand side term of Eq. 5, pH 7.4, $T = 298$ K. The inset shows the % of species, represented by the H8 proton of 5′-GMP, of reactant and product over a time range.

Table 1. Second-order rate constants (k_2) and activation parameters for the reactions of Pt(II) complexes with 5′-GMP and sulfur-bonding nucleophiles according to Eqs. 1–4 at 37 °C in 0.1 M Hepes and at pH of 7.4

L	$k_2^{298}/M^{-1}\ s^{-1}$	$\Delta H_2^{\neq}/kJmol^{-1}$	$\Delta S_2^{\neq}/JK^{-1}\ mol^{-1}$
cis-$[Pt(NH_3)Cl_2]$			
GSH	$(8.45 \pm 0.08) \cdot 10^{-2}$		
5′-GMP	$(2.07 \pm 0.03) \cdot 10^{-3}$		
5′-GMP[a]	$(1.32 \pm 0.04) \cdot 10^{-3}$		
$[Pt(terpy)Cl]^+$			
GSH	$(1.32 \pm 0.04) \cdot 10^{3}$	36 ± 4	−61 ± 14
5′-GMP	$(1.98 \pm 0.08) \cdot 10^{2}$		
L-Cysteine	$(2.13 \pm 0.55) \cdot 10^{3}$		
Thiourea	$(1.52 \pm 0.48) \cdot 10^{4}$		
$[Pt(terpy)(S\text{-cys})]^{2+}$			
Thiourea	1.35 ± 0.02	34 ± 2	−68 ± 7
Thiosulfate	7.03 ± 0.09		
DEDTC	9.30 ± 0.4		
$[Pt(terpy)(N7\text{-guo})]^{2+}$			
L-Cysteine	$(1.56 \pm 0.02) \cdot 10^{2}$	44 ± 2	−110 ± 5
Glutathione	$(1.32 \pm 0.07) \cdot 10^{2}$		
Thiourea	$(2.02 \pm 0.08) \cdot 10^{2}$		
Thiosulfate	$(8.62 \pm 0.18) \cdot 10^{2}$		
DEDTC	$(1.64 \pm 0.18) \cdot 10^{3}$		

[a] Rate constant obtained by ^1H NMR experiments at 298 K.

2.2. Reactions of $[Pt(terpy)Cl]^+$ with 5′-GMP and sulfur donor nucleophiles

The mono-functional $[Pt(terpy)Cl]^+$ and related complexes of the general type $[Pt(terpy)X]^{2+}$ (terpy = 2,2′:6′,2″-terpyridine) are very useful models for studying the ligand substitution reactions of square–planar complexes. The substitution of Pt(II) complexes with the used nucleophiles was investigated as a function of time by following the change in absorbance at a suitable wavelength. Substitution reactions of square–planar d^8 metal complexes are, in general, accepted to proceed via two parallel associative reaction paths.[32] The observed pseudo-first-order rate constants, k_{obsd}, as a function of the total concentration of nucleophile are described by Eq. 6.

$$k_{obsd} = k_1 + k_2[\text{nucleophile}] \quad (6)$$

The solvolysis rate constant k_1, which is independent of L concentration, can be determined from the intercept of the graph of k_{obsd} versus [L]. The second-order rate constants k_2, which involve the formation of the new complex, can be evaluated from the slope of a plot k_{obsd} versus [L].

The kinetic traces gave excellent fits to a single exponential. k_{obsd}, calculated from the kinetics traces, were plotted versus the concentrations of the entering

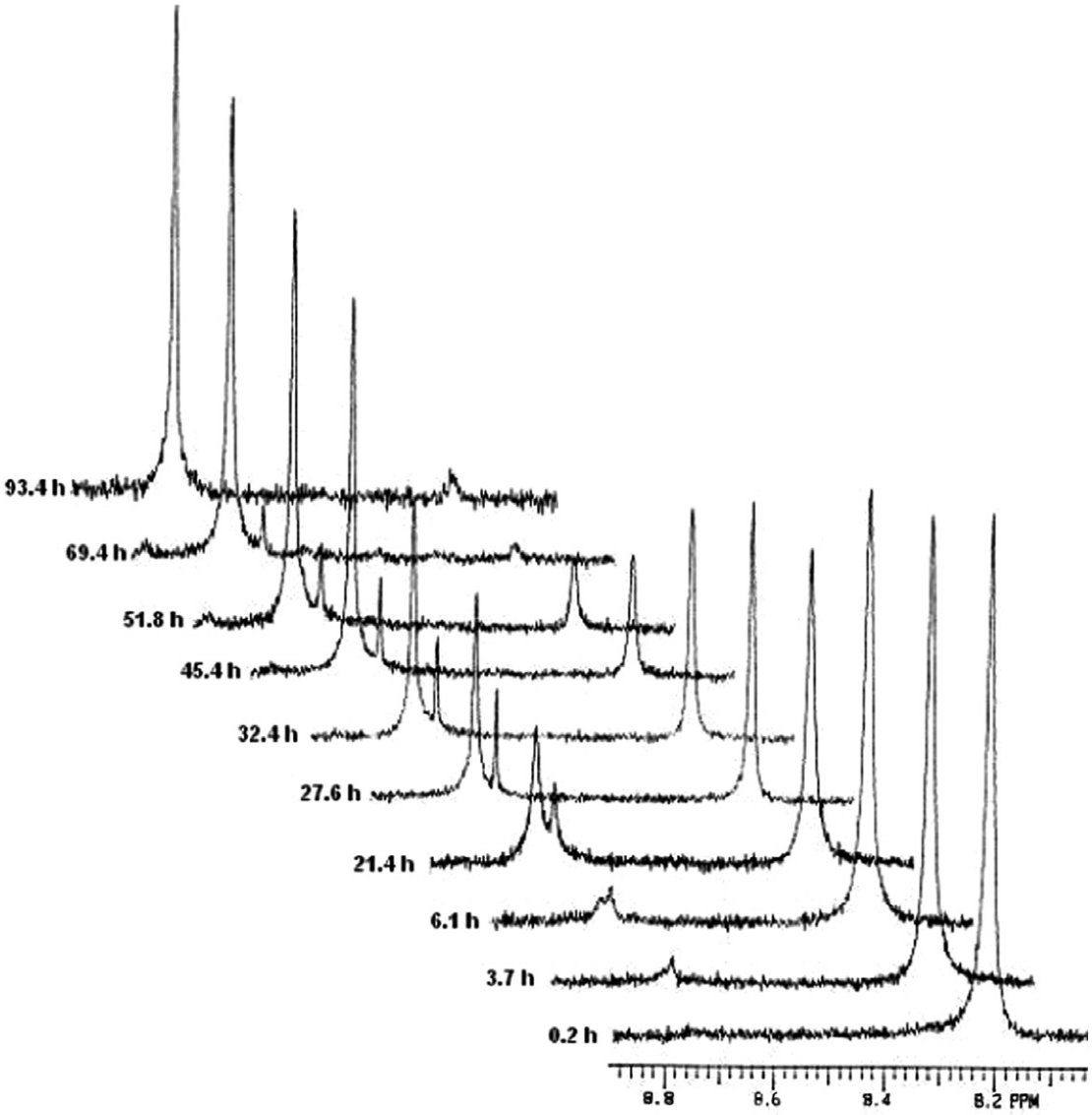


Figure 3. ^{1}H HMR spectra of a solution of 7.5 mM cisplatin and 5′-GMP (7.5 mM) in D_2O at pH 7.4 and 298 K recorded as a function of time.

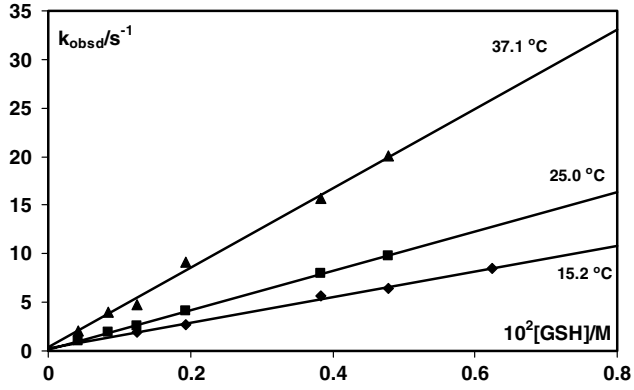


Figure 4. Pseudo-first-order rate constant as a function of concentration of glutathione and temperature for the reaction with $[Pt(terpy)Cl]^+$.

nucleophiles. A linear dependence of k_{obsd} on the nucleophile concentration was observed for all reactions, Figure 4.

Attention has been focused on k_2, which can be measured accurately. Values of k_2 for the displacement of the leaving group (chloride from $[Pt(terpy)Cl]^+$, or L-cysteine from $[Pt(terpy)(S\text{-cyst})]^{2+}$ and guanosine from $[Pt(terpy)(N7\text{-guo})]^{2+}$ complexes) with nucleophiles were obtained by fitting the experimental data to Eq. 6 and are listed in Table 1.

In all cases the substitution reactions are characterized by almost zero values for k_1 (see Fig. 4), in most cases negligible, illustrating that the solvent cannot effectively displace the coordinated nucleophile. Thus, the complex-formation reaction goes almost to completion.

The temperature dependence of these rate constants enabled the calculation of the activation enthalpies and entropies by use of the Eyring equation. Rate constants and activation parameters derived from these experiments are summarized in Table 1.

Guanosine-5′-monophosphate (5′-GMP) can coordinate to metal ions via N1 and N7. Binding through the N7 position in a neutral or weakly acidic medium has been verified.[24,33–36] The kinetic data (Table 1) clearly show that 5′-GMP is very reactive to $[Pt(terpy)Cl]^+$ complex. From a comparison of the reactivity of thiols (L-cysteine and glutathione)with 5′-GMP in the reaction with $[Pt(terpy)Cl]^+$, it can be also concluded that these N-bonding ligand (5′-GMP) cannot compete with thiols. On the other side, in the reactions of $[Pt(terpy)H_2O]^{2+}$ with INO, 5′-IMP, and 5′-GMP at pH 2.5,[24] it has been found that these N-bonding nucleophiles were even more reactive than thiols at pH 1.[22] The preference of these N-bonding nucleophiles over thiols in acidic solutions needs to be addressed. It must be kept in mind that the reactions with thiols have been investigated at pH 1, where all thiols were protonated. On the other hand, at pH 2.5 the N7 sites of INO, 5′-IMP, and 5′-GMP are not protonated. However, at pH of 7.4, thiols are deprotonated (about 90%, see Fig. 2S, Supplementary material), and the N-bonding bases cannot compete with the thiol containing amino acids and peptides.[37] Therefore, binding primarily takes place through the sulfur donor sites. However, for the GSMe system, rapid coordination to the sulfur atom followed by migration to the N7 site of the purine was observed.[19] Similar competition experiments of the $[Pt(dien)Cl]^+$ with a mixture of L-methionine and 5′-GMP also afforded sulfur bound intermediates, followed by the formation of Pt–N7-GMP products.[29] On the other hand, it has been known, and we also demonstrated in the recently published paper that 5′-GMP cannot substitute thiols from Pt–thiolate adduct.[25] These findings could have implications for the mechanism of action of platinum anticancer drugs. Sulfur-bonding ligands have a much higher affinity for Pt(II) complexes than nitrogen-bonding ligands,[29,38] and to diminish the anti-tumor activity of platinum complexes. Moreover, nephrotoxicity has been explained by the Pt–S (GSH) interactions.[7,39]

2.3. Reactions of $[Pt(terpy)(S\text{-}cys)]^{2+}$ with sulfur donor nucleophiles

Several sulfur donor ligands are usually co-administered with platinum drugs to reduce the toxicity.[40,41] Some of them, such as glutathione, L-cysteine, diethyldithiocarbamate, thiosulfate, and thiourea, were used in the study with $[Pt(terpy)(S\text{-}cys)]^{2+}$ and $[Pt(terpy)(N7\text{-}gua)]^{2+}$ complexes.

The $[Pt(terpy)(S\text{-}cys)]^{2+}$complex is unreactive toward nitrogen binding ligands, and cysteine cannot be replaced by N7 5′-GMP.[28] However, very strong sulfur-donor nucleophiles, such as diethyldithiocarbamate, thiosulfate, and thiourea, could reverse the Pt–cysteine bond under our experimental conditions (pH of 7.4). Linear plots of the observed pseudo-first-order rate constants k_{obsd} versus the total concentration of the nucleophile pass almost through the origin. The intercept is very small within the experimental error limits (Fig. 5). Thus no significant solvent or reverse reaction path was observed in the present systems, such that direct

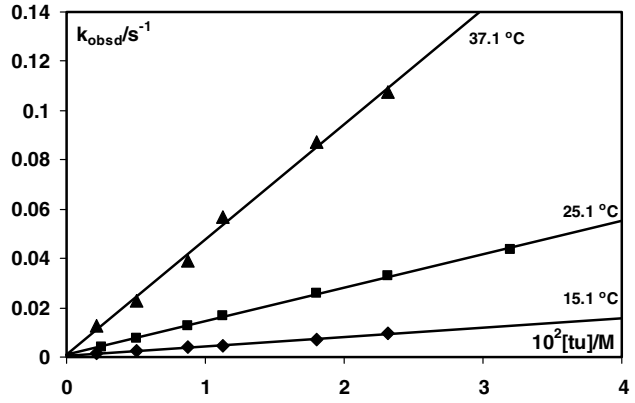


Figure 5. Pseudo-first-order rate constant as a function of concentration of thiourea and temperature for the reaction with $[Pt(terpy)(S\text{-}cys)]^{2+}$ and thiourea.

nucleophilic substitution is the major observed reaction pathway under the selected conditions.

The following rate law can be formulated:

$$k_{obsd} = k_2[\text{nucleophile}] \quad (7)$$

where k_2 is a second-order rate constant for the forward reaction in (3). The second-order rate constants, obtained from linear least-squares analysis of the kinetic data, are summarized in Table 1. The thermal activation parameters are also listed in Table 1. It can be seen that dedtc is the best nucleophile and the order of reactivity is thiourea < thiosulfate < dedtc (Fig. 6). This is in an excellent agreement with previously published results,[24] and also it is in agreement with the results published for the reactions of the *cis*-$[Pt(NH_3)_2Cl_2]$, $[Pt(dien)Cl]^+$, and $[Pd(dien)(GSMe)]^{2+}$ complexes with some rescue agents. It was also found that dedtc is the most effective rescue agent.[42]

The results presented here clearly show that therapeutic nucleophilic agents for platinum drugs, such as diethyldithiocarbamate, thiosulfate and thiourea, may help to displace Pt from Pt–cysteine adducts and in that way could reduce nephrotoxicity.

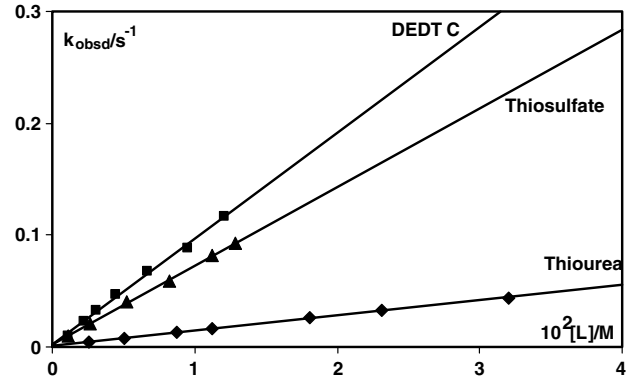


Figure 6. Pseudo-first-order rate constant as a function of concentration of different ligands for the reactions with $[Pt(terpy)(S\text{-}cys)]^{2+}$ at 298 K.

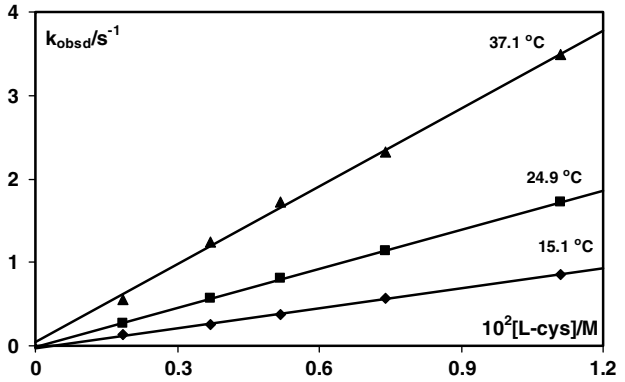


Figure 7. Pseudo-first-order rate constant as a function of concentration of L-cysteine and temperature for the reaction with $[Pt(terpy)(N7\text{-}guo)]^{2+}$.

2.4. Reactions of $[Pt(terpy)(N7\text{-}guo)]^{2+}$ with sulfur donor nucleophiles

It is widely accepted that, once formed, the Pt–nucleobase complexes are inert under mild conditions and in the absence of strong *trans*-labilizing ligands.[43] In contrast, the presence of strong nucleophiles, for instance sulfur-containing biomolecules, could facilitate the dissociation of N-coordinated nucleobases from the Pt complex. In particular, various sulfur-containing molecules have aroused considerable interest owing to their important roles in the biological processing of anticancer platinum drugs.[7]

In the present work, we studied substitution of guanosine from $[Pt(terpy)(N7\text{-}guo)]^{2+}$ by some sulfur-donor nucleophiles which have been used as protecting agents. In all cases, the observed rate constant, k_{obsd}, increased linearly with increasing nucleophile concentration (see Fig. 7). At each temperature, the intercept was small. The second-order rate constants and the activation parameters for the forward reaction in 4 are summarized in Table 1.

The result presented here strongly indicates that all studied sulfur-donor nucleophiles could substitute guanosine from $[Pt(terpy)(guo\text{-}N7)]^{2+}$. From the data it can be seen that dedtc is the strongest nucleophile. Moreover, L-cysteine and tripeptide glutathione are very efficient nucleophiles as well. This observation could be very important since it is already known that glutathione has numerous cellular functions, including the detoxification of chemotherapeutic agents.[31]

From our results we can conclude that the employed rescue or protecting agents such as thiourea, thiosulfate, and diethyldithiocarbamate, can much easily substitute guanosine than L-cysteine from the $[Pt(terpy)X]^{2+}$ complex (X is N7-guo or *S*-cys). This is in excellent agreement with previous investigations, where it has been shown that the Pt–S (cysteine) bond is very stable.[21,24] The thiolate ion is capable of providing a stronger binding affinity owing to its better σ-donating ability. Such a Pt–S bond is considered relatively inert, which may cause the inhibition of the anticancer activity of platinum drugs.

3. Conclusion

In conclusion, it was shown that N-bonding ligand such as 5′-GMP has a high affinity for cisplatin and $[Pt(terpy)Cl]^+$ complex, which may have important biological implications, since the interactions of Pt(II) with DNA are thought to be responsible for the anti-tumor activity of platinum drugs. However, the preference of Pt(II) to coordinate to S-bonding nucleophiles was demonstrated. These results also show that Pt(II)–N7-GMP adducts can easily be converted into Pt–S adducts. It must be noted that the thiols (L-cysteine and glutathione) are a very efficient reagent. This would suggest that glutathione could also displace coordinated bases of DNA in Pt–DNA adducts and thioethers in Pt–S(thioether) products as well,[21,24] forming a very stable Pt–S(thiolate) bond. On the other hand, the Pt–S(thiolate) bond appears to be too stable and cannot be broken by N-bonding 5′-GMP.

Finally, this investigation demonstrated that therapeutic nucleophilic agents such as dedtc, thiosulfate, and thiourea, may assist the dissociation of the Pt–S(cysteine) bond. Our results strongly indicate that dedtc is the most reactive reagent.

4. Experimental

4.1. Synthesis of complexes

The complex $[Pt(terpy)Cl]Cl{\cdot}2H_2O$ was prepared according to a literature method.[44] Chemical analysis, UV–vis, and ^1H NMR spectral data were in good agreement with those obtained in previous preparations. The complexes $[Pt(terpy)(S\text{-}cyst)](ClO_4)_2{\cdot}0.5\ H_2O$ and $[Pt(terpy)(N7\text{-}guo)](ClO_4)_2{\cdot}0.5\ guo{\cdot}1.5\ H_2O$ were prepared as already published.[24] Cisplatin, *cis*-$[Pt(NH_3)_2Cl_2]$, was purchased from Aldrich.

4.2. Chemicals and solutions

Ligand stock solutions were prepared without further purification shortly before use by dissolving the chemicals, L-cysteine (Fluka, Assay > 99.5%), glutathione (Fluka, Assay > 99%), thiourea (Merck, pa), guanosine-5′-monophosphate sodium salt hydrate (Sigma), sodium thiosulfate pentahydrate (Acros), and sodium diethyldithiocarbamate trihydrate (Sigma). D_2O, 99.9%, (Deutero GmbH) is commercially available and used as received. All other chemicals were of the highest purity commercially available and were used without further purification. Hepes buffer (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid) was obtained from Aldrich. Ultra pure water was used in all experiments involving aqueous solutions.

4.3. Instrumentation

Chemical analyses were performed on a Carlo Erba Elemental Analyser 1106. UV–vis spectra were recorded on Shimadzu UV 250 and Hewlett–Packard 8452A diode-array spectrophotometers with thermostated

1.00 cm quartz Suprasil cells. Kinetic measurements were carried out on High-Teck stopped-flow instrument coupled to an online data acquisition system. The temperature was controlled throughout all kinetic experiments to ±0.1 °C. All kinetic measurements were performed under pseudo-first-order conditions, that is, at least a 10-fold excess of the entering nucleophile was used.

The NMR spectra were acquired on a Varian Gemini-200 spectrometer. The measurements were performed with a commercial 5 mm Bruker broadband probe. All chemical shifts are referenced to TSP (trimethylsilylpropionic acid) in D_2O, downfield shifts recorded as positive numbers. All pD measurements were performed at 25 °C. The pH meter was calibrated with Fischer-certified buffer solutions of pH 4.00, 7.00, and 11.00. Meter readings were corrected for the deuterium isotope effect by adding 0.4 U to the display readout. The pD was adjusted with 0.01–0.05 M solutions of NaOD and DCl.

4.4. Kinetics measurements

Spectral changes resulting from the mixing of complex and nucleophile solutions were recorded over the wavelength range 220–450 nm to establish a suitable wavelength at which kinetic measurements could be performed. Reactions were initiated by adding of 0.5 cm^3 solution of cisplatin complex to 2.5 cm^3 of ligand thermostated solution in the UV–vis spectrophotometric cell, and were followed for at least eight half-lives. Reactions of the $[Pt(terpy)Cl]^+$, $[Pt(terpy)(S\text{-cys})]^{2+}$, and $[Pt(terpy)(N7\text{-guo})]^{2+}$ complexes were initiated by mixing equal volumes of the complex and ligand solutions directly in the stopped-flow instruments and were followed for at least eight half-lives. Complex-formation was monitored as an increase in absorbance at 336, 342 or 363 nm under pseudo-first-order conditions. The temperature of the instruments was controlled throughout all kinetic experiments to an accuracy of ±0.1 °C. Hepes (0.1 M) and NaOH were used as a buffer at pH 7.4 and the ionic strength was therefore 0.1 M for the measurements. All kinetic runs could be fitted by a single exponential function, and no subsequent reactions were observed. The observed pseudo-first-order rate constants, k_{obsd}, were calculated as the average value from two to five independent runs. The temperature dependence of k_{obsd} was studied in the interval 15–37 °C. Experimental data are reported in Tables S2–S15 (Supplementary material) and are summarized in Figures 4 and 7.

4.5. NMR measurements

All reactions were carried out in NMR tubes. D_2O was used as a solvent. All chemical shifts are referenced to TSP (trimethylsilylpropionic acid) in D_2O, downfield shifts recorded as positive numbers. For the kinetic measurements the reactions of *cis*-$[Pt(NH_2)_2Cl_2]$(15 mM) with 5′-GMP (in equimolar amount) ^1H NMR spectra were recorded subsequently (the first after 20 min, and the others after a few hours). However, these reactions were monitored after a few days up to three weeks. Experimental data are reported in Table S1 (Supplementary material).

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2007.03.059.

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