Reactions of [PtCl(dien)]Cl with Glutathione, Oxidized Glutathione and *S*-Methyl Glutathione. Formation of an S-bridged Dinuclear Unit

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Abstract

The reaction of the monofunctional platinum compound [PtCl(dien)]Cl with the tripeptide glutathione (GSH), oxidized glutathione (GSSG) and *S*-methyl glutathione (GS-Me) has been investigated by ^{1}H, ^{13}C and ^{195}Pt magnetic resonance spectroscopy and by potentiometric titrations. It appears that platinum binds with a high degree of specificity to the GSH sulfhydryl group. The reaction of platinum with GSH proceeds in two steps. In the first step only one platinum binds to the sulfur atom and, in the second step, another $[Pt(dien)]^{2+}$ unit binds to $[Pt(dien)GS]^{+}$ forming an S-bridged dinuclear unit $[\{Pt(dien)\}_2GS]^{3+}$. The rate of the first binding step is pH-dependent, whereas the rate of the second step is not. At pH < 7 the rate of the first binding step is slow compared to the rate of the second binding step. At pH > 10, on the other hand, the rate of the first binding step is faster than the rate of the second binding step. Consequently, at pH < 7 one can only isolate the $[\{Pt(dien)\}_2GS]^{3+}$ complex. In the presence of free GSH, at pH > 7, one $[Pt(dien)]^{2+}$ unit of $[\{Pt(dien)\}_2GS]^{3+}$ dissociates forming $[Pt(dien)GS]^{+}$. The mechanism of the pH-dependent rate of the first platinum binding step and the ligand-exchange reaction are discussed. GSSG reacts with $[Pt(dien)]^{2+}$, also forming the S-bridged dinuclear unit $[\{Pt(dien)\}_2GS]^{3+}$, probably through a redox disproportionation reaction with a catalytic function of [PtCl(dien)]Cl. GS-Me reacts with $[Pt(dien)]^{2+}$ forming the S-coordinated $[Pt(dien)GS\text{-}Me]^{2+}$. $[Pt(dien)GS\text{-}Me]^{2+}$ exists as a pair of diastereomers due to different configurations about sulfur. The rate of the inversion of configuration at the coordinated sulfur atom is slow on the NMR timescale.

Introduction

It is generally accepted that interactions of cisplatin (*cis*-diamminedichloroplatinum, cis-Pt) with DNA are responsible for its observed antitumor activity [1, 2]. Under *in vivo* conditions many other ligands in the cytoplasm are also able to react with cis-Pt. Especially sulfur-containing molecules are known to be reactive [3]. The binding of cis-Pt to protein-bound sulfhydryl groups is thought to be responsible for the observed toxic side-effects [4]. Therefore it is of great importance to study these interactions in detail. Glutathione (GSH) (**1**), a cysteine-containing tripeptide, which is present in the cytoplasm (at concentrations of 0.5–10 mM), is a likely candidate for biologically relevant reactions and can serve as a useful model compound for sulfur-containing proteins. Therefore a systematic study was undertaken of the interactions between platinum amine compounds and sulfur-containing ligands like GSH. A proposed hypothesis about the biological role of GSH in the working mechanism of platinum anti-cancer drugs states that GSH can act as an inactivator of platinum complexes, thereby preventing binding to DNA [5, 6] and also that GSH can act as a trapping agent of DNA–Pt monoadducts, thereby preventing further reaction to form the cytotoxic bifunctional adducts [6–8]. This makes a binding study of platinum compounds with GSH highly relevant.

From a literature study it appears that some disagreement exists about the possible interactions between cis-Pt and GSH [9–12]. This is mainly the result of the large *trans*-labilizing effect of a coordinated sulfur [13]. Consequently, the coordination complexes formed will be relatively unstable. To avoid this problem it was decided to investigate the relatively simple, monofunctional analog [PtCl(dien)]Cl (**2**), which is often used to represent the first binding step of cis-Pt [14]. This compound is more stable than cis-Pt and therefore will be less susceptible to the *trans*-labilizing effect of a coordinated sulfur [13]. The aim of the present study was to gain some insight into the binding properties of the non-antitumor active compound [PtCl(dien)]Cl, which will hopefully lead to a better understanding of the binding of cis-Pt to GSH. Part of this study was briefly mentioned in a preliminary communication [15]. The results will be compared with earlier studies in which the

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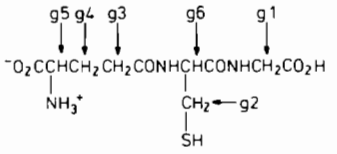
binding of CH_3Hg^+ to GSH has been investigated [16–24].



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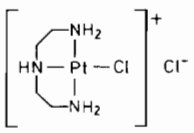

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Experimental

Chemicals

The platinum compound [PtCl(dien)]Cl (dien = diethylenetriammine) was prepared according to Watt and Cude [25]. Glutathione, oxidized glutathione and *S*-methyl glutathione were obtained from Sigma Chemicals and used without further purification.

pH Measurements

All pH measurements were performed at 298 K. The pH meter was calibrated with Fisher certified buffer solutions of pH 4.00, 7.00 and 10.00.

NMR Measurements

The ^1H and ^{195}Pt (at 64.4 MHz) NMR spectra were recorded with a Bruker WM 300 spectrometer. The ^{13}C (at 50.1 MHz) NMR spectra were recorded with a JEOL 200 spectrometer. D_2O was used as a solvent. References were Na_2PtCl_6 for ^{195}Pt (external), TMA (tetramethylammonium nitrate) for ^1H and dioxane for ^{13}C. ^1H and ^{13}C NMR spectra were recorded at 295 K. For the ^{13}C and ^{195}Pt NMR measurements concentrations of 100–200 mM of substrate were used, while for the ^1H NMR measurements concentrations of 5 mM were sufficient. The ^{195}Pt-decoupled ^1H NMR spectrum was recorded at 310 K. Pulse widths of 6 μs and a relaxation delay of 0.5 s produced the ^{195}Pt NMR spectra at pH 7 after 1000 transients. For monitoring the pH-dependent chemical shift behavior of the ^1H and ^{13}C signals of the products, the pH was adjusted with 0.1 and 1 M solutions of NaOD and DCl. The pH, reported as pH*, has not been corrected for the deuterium isotope effects.

Reactions

All reactions were carried out in NMR tubes at a concentration of 5 mM substrate over the pH range 2–12. All reactions were followed using ^1H NMR spectroscopy at 295 K. Reaction products were purified by gel permeation chromatography (Sephadex G-10, Pharmacia). The number of moles of hydroxide ions consumed due to the formation of the complexes was determined by potentiometric titrations with 0.1 N NaOH as a titrant.

Results and Discussion

General Observations

The proton NMR resonances of free [PtCl(dien)]Cl in the reaction mixture were used as an indication of whether the reaction was complete or not. The formation of the adducts was independent of the order of addition of the reaction components. At pH < 7 a [PtCl(dien)]Cl-to-GSH ratio of 2:1 was required to bind all available GSH. This already suggested a product in which two $[Pt(dien)]^{2+}$ units are bound to GSH. At pH > 10, on the other hand, a [PtCl(dien)]Cl-to-GSH ratio of only 1:1 was required to bind all available GSH. The adduct formed was different from the one formed at low pH. Moreover, when a second equivalent [PtCl(dien)]Cl was added to this 1:1 adduct (at any pH between 2 and 12) the same 2:1 adduct was formed as described above for pH < 7. From this result it can be concluded that the 1:1 adduct isolated at pH > 10 is an intermediate in the formation of the 2:1 adduct. At pH < 7 the 1:1 adduct could not be isolated, due to a very fast second Pt-binding step compared to the first Pt-binding step.

Characterization of the Complexes

The pH dependence of the chemical shift of the protons g2 and g6 of GSH and the two Pt complexes is depicted in Fig. 1. As can be seen, the chemical shifts of the protons g2 and g6 of the two Pt complexes are independent of pH. This indicates that a $[Pt(dien)]^{2+}$ unit coordinates in both complexes to the ionized sulfhydryl group. The chemical shift differences of the protons g1, g3, g4 and g5, which are due to the complexation of GSH with $[Pt(dien)]^{2+}$, are all smaller than 0.1 ppm (see Table I). Furthermore, the chemical shifts of the protons g1, g3, g4 and g5 depend on pH, possibly due to protonation/deprotonation equilibria of the amino

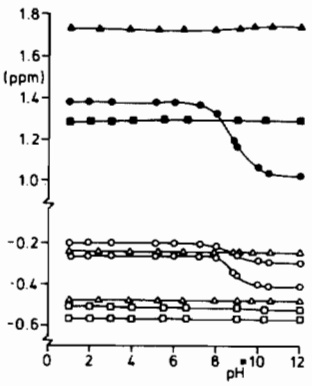

Fig. 1. Chemical shifts (δ) of the two g2 and the g6 protons of free GSH (○), 1:1 Pt(dien)–GSH (□) and 2:1 Pt(dien)–GSH (△) as a function of pH* at 295 K. Chemical shifts are in ppm relative to TMA. Open symbols represent g2, closed symbols represent g6.

TABLE I. 300 MHz ^{1}H NMR Data of GSH, GS-Me and their Complexes with [PtCl(dien)]Cl (Chemical Shifts (δ) are in ppm Relative to TMA at pH* 5.0 at 295 K)

	g1	g2	g3	g4	g5	g6	Me
GSH	0.60	−0.21 −0.27	−0.63	−1.02	0.59	1.39	
[Pt(dien)GS]$^+$	0.67	−0.51 −0.57	−0.63	−1.02	0.61	1.30	
[{Pt(dien)}$_2$GS]$^{3+}$	0.69 0.60	−0.48 −0.24	−0.62	−1.02	0.59	1.72	
GS-Me	0.61 0.58	−0.15 −0.34	−0.64	−1.03	0.58	1.40	−1.06
[Pt(dien)GS-Me]$^{2+}$	0.66 0.59	[a] [a]	−0.62	−1.03	0.58	1.77	−0.59

[a]Resonances overlapping with the [PtCl(dien)]Cl resonances.

group and the two carboxyl groups and confirming that no coordination has occurred at these groups.

The remaining and most important question is to which atom the second [Pt(dien)]$^{2+}$ unit is coordinated in [{Pt(dien)}$_2$GS]$^{3+}$. The chemical shifts of the protons g2 and g6 of [{Pt(dien)}$_2$GS]$^{3+}$ are downfield compared to [Pt(dien)GS]$^+$. In principle, coordination of the second [Pt(dien)]$^{2+}$ unit could therefore be at either the deprotonated amide nitrogen, the carbonyl oxygen, or the already platinated sulfur atom of the cysteine residue.

The results of the potentiometric titration show that the number of moles of OH^-, consumed per mole of [{Pt(dien)}$_2$GS]$^{3+}$, is 0.9 ± 0.1. A ratio of 2.0 would correspond to a doubly deprotonated [{Pt(dien)}$_2$GS]$^{2+}$, suggesting no coordination of the second [Pt(dien)]$^{2+}$ unit to the deprotonated amide nitrogen. This is consistent with the fact that no pH decrease is observed in the reaction of [Pt(dien)GS]$^+$ with one equivalent [PtCl(dien)]Cl.

To distinguish between the two remaining possible sites, *i.e.* the sulfur atom and the carbonyl oxygen of the cysteine residue, ^{13}C NMR was used. Due to a concentration-dependent decomposition reaction of [Pt(dien)GS]$^+$ at pH < 4, yielding several decomposition products including free dien, it was not possible to obtain ^{13}C NMR spectra at pH < 4 as was observed for a related Pd–amine sulfite complex [26]. At low concentration of [Pt(dien)GS]$^+$ (5 mM) the compound is stable for about 5 h at pH < 4. The pH-dependence of the chemical shift of the carbon atoms cys-CONH, cys-C_α and cys-C_β of GSH and the two complexes is depicted in Fig. 2.

By a similar reasoning as used to interpret the data in Fig. 1, it is concluded that in both complexes a [Pt(dien)]$^{2+}$ unit is coordinated to the ionized sulfhydryl group. The chemical shifts of the carbon atoms cys-CONH (1.7 ppm) and cys-C_α (1.8 ppm) of [{Pt(dien)}$_2$GS]$^{3+}$ are upfield, whereas

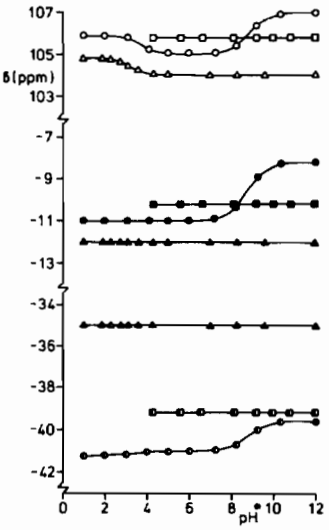

Fig. 2. ^{13}C Chemical shifts (δ) of the three cysteinyl carbons of GSH (○), [Pt(dien)GS]$^+$ (□) and [{Pt(dien)}$_2$GS]$^{3+}$ (△) as a function of pH* at 295 K. Chemical shifts are in ppm relative to dioxane. Open symbols represent cys-CONH, closed symbols represent cys-C_α and half-open symbols represent cys-C_β.

the chemical shift of the carbon atom cys-C_β (4.2 ppm) is downfield, compared to [Pt(dien)GS]$^+$. The resonances of the other seven carbon atoms show nearly the same chemical shift titration behavior for free GSH as for the two complexes (data not shown). Platinum coordination is likely to have the largest influence on the chemical shift of the carbon atom which is the nearest to the coordination site. Therefore it must be concluded that the second [Pt(dien)]$^{2+}$ unit in [{Pt(dien)}$_2$GS]$^{3+}$ is also coordinated to the sulfhydryl group.

The coordination of the second [Pt(dien)]$^{2+}$ unit results in a downfield shift (0.42 ppm) of the g6 proton (see Table I). To find out whether this

observation is unique or not, the $[Pt(dien)]^{2+}$ binding to $[Pt(dien)GS]^{+}$ was compared with the binding of $[Pt(dien)]^{2+}$ to GS-Me (see Table I). The large downfield shift of 0.47 ppm of the methyl group attached to the sulfur atom clearly indicates that platinum coordinates to the sulfur atom. Again a large downfield shift (0.37 ppm) of the g6 proton upon coordination of $[Pt(dien)]^{2+}$ to GS-Me is observed. The large downfield shift of the g6 proton is probably the result of the inductive effect due to Pt^{2+} binding.

The results of the ^{13}C NMR study of GS-Me and $[Pt(dien)GS\text{-}Me]^{2+}$ confirm the coordination of $[Pt(dien)]^{2+}$ to the sulfur atom. The chemical shifts of the carbon atoms $\underline{C}OCHCH_2SCH_3$ (1.9 ppm) and $\underline{C}HCH_2SCH_3$ (1.8 ppm) of $[Pt(dien)GS\text{-}Me]^{2+}$ are upfield, whereas the chemical shifts of the carbon atoms $\underline{C}H_2SCH_3$ (5.5 ppm) and $CH_2S\underline{C}H_3$ (6.4 ppm) are downfield, compared to GS-Me. The resonances of the other seven carbon atoms show nearly the same chemical shift titration behavior for GS-Me as for $[Pt(dien)GS\text{-}Me]^{2+}$ (data not shown).

Theoretically the coordination of $[Pt(dien)]^{2+}$ to the sulfur atom of GS-Me would result in two different configurations about sulfur and consequently this would lead to a pair of diastereomers. ^1H NMR could not distinguish between them, although the signal of the g6 proton shows some broadening at 277 K. Even the proton signal of the CH_3 substituent on the sulfur shows no doubling at 277 K. Apparently, ^1H NMR is not sensitive enough for the subtle differences between the pair of diastereomers. On the contrary, ^{13}C NMR shows clear doubling of the signals $\underline{C}HCH_2SCH_3$ ($\Delta = 0.5$ ppm), $\underline{C}H_2SCH_3$ ($\Delta = 0.5$ ppm) and $CH_2S\underline{C}H_3$ ($\Delta = 0.8$ ppm) at 277 K (the signals of $\underline{C}H_2SCH_3$ and $CH_2S\underline{C}H_3$ are depicted in Fig. 3). All other ^{13}C signals further away from the Pt-coordination site still remain singlets even at 277 K. In other words, only the carbon atoms nearest to the platinum coordination site are sensitive to the small differences between the pair of diastereomers. This diastereomeric splitting is one of the few examples of detectable diastereomers originating from unidentate thioether ligands [27–29]. In Fig. 3 the inversion of the pyramidal configuration at the platinum coordinated sulfur atom is depicted by using variable-temperature ^{13}C NMR spectroscopy [30].

Although the structural changes between the pair of diastereomers must be quite small, there appears to be some chiral discrimination, since the ratio between the two sets of peaks is about 2:3 at low temperatures. At this moment we cannot relate this ratio to particular structural differences. Kostic *et al.* have elegantly shown that ^{195}Pt NMR spectroscopy is very useful to distinguish between diastereomers in platinum(II) thioether complexes

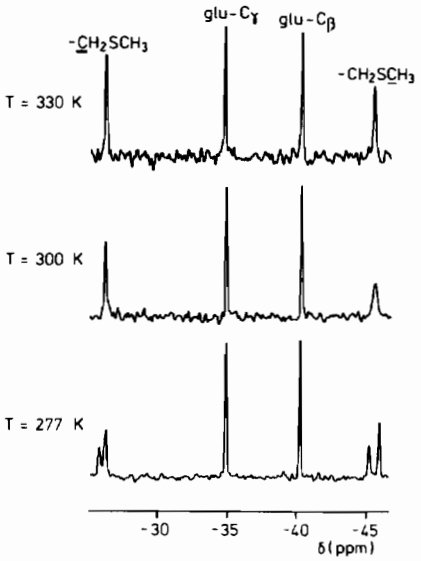

Fig. 3. ^{13}C NMR spectra of the carbon atoms $-\underline{C}H_2SCH_3$, $-CH_2S\underline{C}H_3$, glu-C_β and glu-C_γ of $[Pt(dien)GS\text{-}Me]^{2+}$ at three temperatures ($pH^* = 5$), showing the appearance of diastereomers. Chemical shifts (δ) are in ppm relative to dioxane.

[28, 29]. Unfortunately, our attempt to use ^{195}Pt NMR to distinguish between the pair of diastereomers was not successful. Only a single broad signal could be observed (width at half-height is 250 Hz). The two expected resonances are probably not resolved due to the broadness of the signal. Apparently, the fast relaxation of the ^{14}N nucleus in the dien ligand causes the severe broadening of the ^{195}Pt resonance [31].

The ^{195}Pt NMR spectra of $[Pt(dien)GS]^{+}$, $[\{Pt(dien)\}_2GS]^{3+}$ and $[Pt(dien)GS\text{-}Me]^{2+}$ all show a singlet at $\delta = -3152$, $\delta = -3180$ and $\delta = -3364$ ppm respectively (this also implies that in $[\{Pt(dien)\}_2GS]^{3+}$ the two $[Pt(dien)]^{2+}$ units are equivalent), shifted upfield from free $[PtCl(dien)]Cl$ (-2727 ppm). The ^{195}Pt satellites of ^1H resonances are very broad at high magnetic field and therefore difficult to assign [32]. It is possible to eliminate this broadening by recording the ^1H NMR spectrum of $[\{Pt(dien)\}_2GS]^{3+}$ selectively irradiated at the ^{195}Pt resonance (Fig. 4A). The difference between this ^{195}Pt decoupled ^1H NMR spectrum (Fig. 4A) and the ^{195}Pt coupled ^1H NMR spectrum (Fig. 4B) should give information about the $^{195}Pt-^1H$ couplings (Fig. 4C). The peaks of Fig. 4C must derive from protons which exhibit a $^{195}Pt-^1H$ coupling. As can indeed be seen, only the protons g2 and g2′ of GSH exhibit a $^{195}Pt-^1H$ coupling. This is in agreement with coordination of the two $[Pt(dien)]^{2+}$ units only to the sulfhydryl group. The remaining signals in Fig. 4C are all from ^1H resonances of coordinated $[Pt(dien)]^{2+}$ protons.

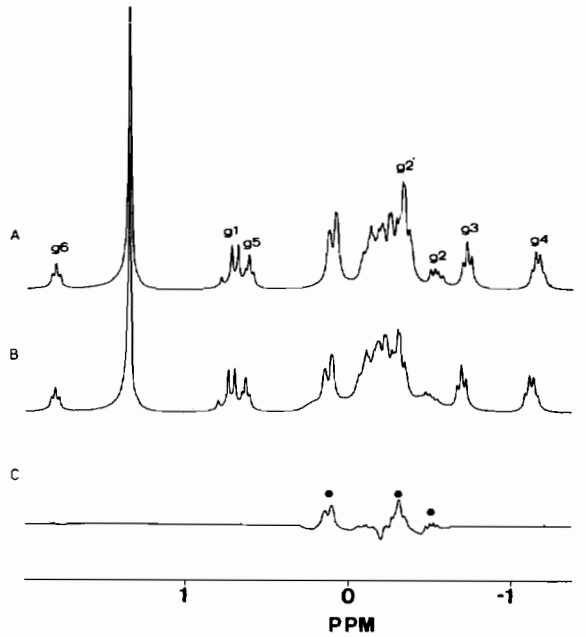

Fig. 4. (A) ^{195}Pt-decoupled ^{1}H NMR spectrum of $[\{Pt(dien)\}_2GS]^{3+}$. (B) ^{195}Pt-coupled ^{1}H NMR spectrum of $[\{Pt(dien)\}_2GS]^{3+}$. (C) The difference between (A) and (B). The protons which exhibit a $^{3}J(^{195}Pt-^{1}H)$ coupling are indicated by ●. All spectra were obtained at 310 K and pH* 7 without Gaussian multiplication.

Very surprisingly, GSSG also reacts with $[Pt(dien)]^{2+}$, producing $[\{Pt(dien)\}_2GS]^{3+}$ together with another product. This reaction is slow ($t_{1/2}$ = 10 days at pH 4, T = 293 K) compared to the reaction of GSH with $[Pt(dien)]^{2+}$. Probably this is a redox disproportionation reaction in which [PtCl(dien)]Cl acts as a catalyst. A related reaction has already been described for Ag^+ by Cecil and McPhee [33], and is represented by eqn. (1):

$$2GSSG + 3Ag^+ + 2H_2O \longrightarrow 3GSAg + GSO_2H + 3H^+ \quad (1)$$

The corresponding reaction of GSSG with $[Pt(dien)]^{2+}$ can then be represented by eqn. (2):

$$2GSSG + 6[Pt(dien)]^{2+} + 2H_2O \longrightarrow 3[\{Pt(dien)\}_2GS]^{3+} + GSO_2H + 3H^+ \quad (2)$$

This reaction shows that platinum is able to catalyze the breaking of S–S bridges and it is important to realize this when investigating interactions between platinum and proteins.

Mechanistic Considerations

To illustrate that the reaction rate of the first Pt-binding to GSH is pH-dependent, a competition reaction was set up, as represented by Scheme 1.

At pH < 7, only $[Pt(dien)GS]^+$ reacts with $[Pt(dien)]^{2+}$, leaving unreacted GSH in the reaction mixture. At pH > 10, on the other hand, GSH reacts with $[Pt(dien)]^{2+}$ leading to $[Pt(dien)GS]^+$. This clearly indicates that the reaction rate of the first Pt-binding step is pH-dependent and that $[Pt(dien)GS]^+$ is the actual intermediate in the formation of $[\{Pt(dien)\}_2GS]^{3+}$ (pH range 2–12). The $t_{1/2}$ (T = 295 K) values as determined by NMR of the two Pt-binding steps are indicated in Scheme 2.

It seems likely that the pH-dependence of the first Pt-binding step stems from a combination of factors. At pH < 7 the first binding step causes a conformational change of GSH. This metal-induced conformational change makes the coordinated sulfhydryl group more accessible for the binding of the second $[Pt(dien)]^{2+}$ unit. Furthermore, at pH < 7 the first binding step consists of a rather slow platinum-promoted deprotonation of the –SH group combined by binding at the GS^-. At pH > 10, on the other hand, the sulfhydryl group is already ionized, allowing very rapid binding to platinum and also allowing isolation of $[Pt(dien)GS]^+$. Finally, it should be realized that metal–thiolate complexes are still good nucleophiles due to the remaining electron density on the sulfur atom [34, 35]. The

$$GSH + [Pt(dien)GS]^+ + [Pt(dien)]^{2+} \underset{pH > 10}{\overset{pH < 7}{\rightleftharpoons}} \begin{matrix} GSH + [\{Pt(dien)\}_2GS]^{3+} \\ [Pt(dien)GS]^+ + H^+ \end{matrix}$$

1 : 1 : 1

Scheme 1.

$$GSH \xrightarrow[-H^+]{+[Pt(dien)]^{2+}} [GS–Pt(dien)]^+ \xrightarrow{+[Pt(dien)]^{2+}} \left[GS\begin{matrix}\diagup Pt(dien) \\ \diagdown Pt(dien)\end{matrix}\right]^{3+}$$

first step: $t_{1/2}$ = 7 h (pH < 7); $t_{1/2}$ < 1 min (pH > 10)

second step: $t_{1/2}$ = 15 min (pH < 7); $t_{1/2}$ = 15 min (pH > 10)

Scheme 2.

chemical shifts of the ^1H resonances of the cysteine residue of $[Pt(dien)GS]^+$ are shifted upfield compared to free GSH (see Table I), suggesting that the electron density at the sulfur atom has indeed increased, and that the nucleophilicity of $[Pt(dien)GS]^+$ might therefore be larger than normally observed in thiolate complexes. An example of the good nucleophilicity of $[Pt(dien)GS]^+$ is shown by the reaction in the presence of excess CH_3I, represented by the general eqn. (3):

$$[Pt(dien)GS]^+ + CH_3I \xrightarrow{pH\ 7} [Pt(dien)GS\text{-}Me]^{2+} + I^- \quad (3)$$

$[Pt(dien)GS\text{-}Me]^{2+}$ is indeed formed; this is the same compound as that formed between GS-Me and $[Pt(dien)]^{2+}$. $[\{Pt(dien)\}_2GS]^{3+}$ and GS-Me are formed through a side reaction, which can also be carried out separately, as represented by eqn. (4):

$$[Pt(dien)GS]^+ + [Pt(dien)GS\text{-}Me]^{2+} \xrightarrow{pH\ 7} [\{Pt(dien)\}_2GS]^{3+} + GS\text{-}Me \quad (4)$$

Again this reaction shows the unusual reactivity of $[Pt(dien)GS]^+$.

$[\{Pt(dien)\}_2GS]^{3+}$ is a very stable compound over the pH range 2–12. Only at pH > 7, in the presence of free GSH, one $[Pt(dien)]^{2+}$ unit dissociates forming $[Pt(dien)GS]^+$, as indicated in eqn. (5):

$$[\{Pt(dien)\}_2GS]^{3+} + GS^- \xrightarrow{pH > 7,\ T = 295\ K} 2[Pt(dien)GS]^+ \quad (5)$$

This reaction ($t_{1/2}$ = 30 min at pH 11) probably proceeds through a bimolecular displacement of complexed GSH by GS^- analogous of the CH_3Hg(II)–GSH system [19].

The main product of Pt–DNA interactions are Pt–guanosine complexes [36]. Nevertheless, GSH is also a likely candidate for biologically relevant reactions, as shown by the fact that up to a ratio of 10 to 1 of 5′-GMP/GSH, $[Pt(dien)]^{2+}$ only binds to GSH. So, GSH could principally act as an inactivator of platinum complexes and thereby prevent binding to DNA [5, 6].

Hg(II) has, like Pt(II), high binding affinities to RSH ligands. Its coordination chemistry is therefore comparable, although the complexes of Pt(II) are kinetically far less labile than their Hg(II) counterparts. $[Pt(dien)]^{2+}$ in particular is comparable to CH_3Hg^+ (the high cellular toxicity of CH_3Hg^+ is probably the result of interactions between CH_3Hg^+ and RS^- groups [18]), because both are monofunctional coordination compounds [37]. The binding chemistry of CH_3Hg^+ to GSH has already been the subject of extensive research by Rabenstein *et al.* [16–24]. Their main conclusions are as follows. CH_3Hg^+ binds to the ionized sulfhydryl group over the pH range 0–14 [16, 17, 19]. Below pH 4, in the presence of an excess CH_3Hg^+, a second CH_3Hg^+ coordinates to the RS^- group, which above pH 4 moves to the amino group [17]. The rate of the CH_3Hg^+ interchange of the various species is fast on the NMR time-scale [19]. Our results show that there are differences and similarities between the binding of $[Pt(dien)]^{2+}$ to GSH and the CH_3-Hg(II)–GSH system and therefore the mechanism of the toxic properties of both compounds can—to some extent—be related to each other.

Both $[\{Pt(dien)\}_2GS]^{3+}$ and $[Pt(dien)GS\text{-}Me]^{2+}$ can be considered as model compounds for Pt–methionine interactions in proteins. Although Pt-(dien)–GS complexes are more stable than their Hg(II) counterparts, it has been shown in eqns. (4) and (5) that it is possible for nucleophiles to abstract Pt(dien) from the RS^- group. Therefore therapeutic nucleophilic agents, like thiourea and diethyldithiocarbamate (DDTC), should be able to restore the original structure of the proteins (initially distorted by, e.g., Pt–methionine binding) as in eqns. (4) and (5). The toxic side-effects of Pt compounds are reduced in this way.

Conclusions

The present paper describes the first stable platinum complexes formed with GSH. Over the pH range 2–12 $[Pt(dien)]^{2+}$ has a high affinity for the sulfhydryl group, forming an S-bridged dinuclear unit. Surprisingly, at low pH the rate of the second platinum-binding step is fast compared to the rate of the first platinum-binding step. There is release of $[Pt(dien)]^{2+}$ units of $[\{Pt(dien)\}_2GS]^{3+}$ and $[Pt(dien)GS\text{-}Me]^{2+}$, which shows the ability of therapeutic agents to counteract the tendency of Pt compounds to bind to exposed methionine-sulfurs of proteins. From the formation of a dinuclear unit with the relatively simple $[PtCl(dien)]Cl$, it can be concluded that similar reactions with cis-Pt are likely to play a role in the toxic (e.g. for kidneys) action of this compound. However, such reactions are predicted to be quite complicated. Further studies are ongoing with simultaneous binding possibilities to nucleic acid fragments.

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