Platinum−Thioether Bonds Can Be Reverted by Guanine−N7 Bonds in Pt(dien)$^{2+}$ Model Adducts

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To study the possible reversibility of platinum−protein binding, the platinum−methionine and platinum−cysteine model adducts *cis*-[Pt(NH$_3$)$_2$(GSMe)$_2$]$^{2+}$, [Pt(dien)(GSMe)]$^{2+}$, [Pt(dien)(GS)]$^{+}$, and {[Pt(dien)]$_2$(GS)}$^{3+}$ (GSH = glutathione, GSMe = *S*-methylglutathione) have been reacted with 5′-AMP and 5′-GMP and d(GpG) under a variety of reaction conditions. Only 5′-GMP and d(GpG) were found to substitute GSMe (a Pt−thioether bond) from [Pt(dien)(GSMe)]$^{2+}$, forming [Pt(dien)(GMP−N7)]$^{2+}$ and {Pt(dien)[d(GpG)-N7)]}$^{2+}$, respectively. Reacting the GSMe diadduct of cisplatin [*cis*-Pt(NH$_3$)$_2$(GSMe)$_2$]$^{2+}$ with GMP revealed that the N7 of 5′-GMP can also substitute GSMe on cisplatin, forming *cis*-[Pt(NH$_3$)$_2$(GSMe)(GMP−N7)]$^{2+}$ and the GMP diadduct *cis*-[Pt(NH$_3$)$_2$-(GMP−N7)$_2$]$^{2+}$. The platinum−cysteine model adducts [Pt(dien)(GS)]$^{+}$ and {[Pt(dien)]$_2$(GS)}$^{3+}$, however, did not react with 5′-AMP or 5′-GMP at 37 °C. Kinetic experiments have revealed that the intermolecular rearrangement reaction of Pt(dien)$^{2+}$ from thioether sulfur to 5′-GMP is second order and that the reaction is slow at ambient temperature ($t_{1/2}$ = 179 h). At 35 °C the reaction proceeds with a reaction rate ($t_{1/2}$ = 31 h), which appears as a relevant rate for in vivo processes. The obtained results are discussed in relation to the antitumor activity and toxicity of platinum complexes. The data suggest that the existence of a drug reservoir is limited to platinum−thioether-type adducts and that the observed rearrangement only occurs with the N7 of guanine and not with adenine. Consequently, new platinum amine chelate complexes in which a thioether function is present as a leaving group might be successful as platinum antitumor compounds exhibiting reduced toxic side effects.

Introduction

It is now generally accepted that the interactions of cisplatin [*cis*-PtCl$_2$(NH$_3$)$_2$] with DNA are responsible for cisplatin's antitumor activity.[1,2] DNA, however, is not the only cellular component capable of reaction with platinum compounds. Sulfur-containing biomolecules, such as certain proteins (a.o. metallothioneins), amino acids (e.g., methionine), and peptides such as glutathione (GSH), are known to be − both in vivo[3] and in vitro[4] − highly reactive toward cisplatin and other platinum compounds. These interactions are the likely origin of undesired phenomena, like inactivation[5] of cisplatin, development of resistance[6] toward cisplatin, and nephrotoxicity.[7] On the other hand, platinum−sulfur adducts have been postulated to be a drug reservoir[8] for platination at DNA and may act as an activated form of platinum compounds with slowly hydrolyzing leaving groups.[9] In these cases platinum−sulfur adducts may function as intermediates that are eventually transformed into platinum−DNA adducts.

Earlier studies on the competition in platinum binding between sulfur donor atoms and reactive nucleobases using *S*-guanosyl-L-homocysteine (SGH)[10] as a model compound revealed for the first time that the N7 of guanine can replace a sulfur donor atom in a platinum−thioether adduct. In this model system, the initially formed [Pt(dien)(SGH-*S*)]$^{2+}$ rearranges intramolecularly at pH values just below 7, into [Pt(dien)(SGH-N7)]$^{2+}$. It is remarkable that this apparent intramolecular rearrangement only occurs with SGH and does not occur with *S*-adenosyl-L-homocysteine.[11] Subsequent studies, including competition experiments using the nucleopeptide Met-d(TpG),[12] have indicated[12b] that even an intermolecular rearrangement can occur.

To investigate the intermolecular rearrangement and the observed specificity for guanine in more detail, the possible reversibility of platinum−sulfur bonds in proteins was studied by using a model system allowing only intermolecular competi-

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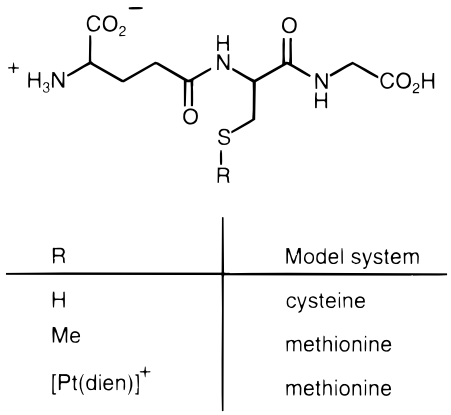


R	Model system
H	cysteine
Me	methionine
$[Pt(dien)]^+$	methionine

Figure 1. Adducts of $[Pt(dien)]^{2+}$ with GSH and GSMe as a model system for Pt−cysteine and Pt−methionine adducts within a protein.

tion. The adducts of $[Pt(dien)Cl]^+$ with GSH and *S*-methylglutathione (GSMe) (Figure 1) were selected as representing the respective Pt−cysteine and Pt−methionine adducts within a protein. Monofunctional $[Pt(dien)]^{2+}$ was chosen as the Pt amine; this is a species with only one leaving group, and therefore mimics the first binding step of cisplatin to biomolecules.[13] Moreover, amine release as a possible consequence of the trans effect of S-donor ligands is avoided because of the stability of the dien chelate.[14] Previous experiments[15] have shown that results obtained with this model system can be extrapolated to the stability of adducts formed by reaction of cisplatin with GSH and GSMe. In addition, GSH not only serves as a model for reactions with Pt−cysteine in proteins; also GSH itself, or its oxidized form GSSG (with cytoplasm concentrations of 0.5−10 mM), is a very likely candidate for in vivo reaction with platinum compounds.

In a study by Lempers and colleagues,[15b] the reactivity of $[Pt(dien)Cl]^+$ and $[Pt(dien)(H_2O)]^{2+}$ toward GSH, GSMe, and 5′-GMP has been investigated. The main aim of this study was to see whether GSMe and GSH bind directly to $[Pt(dien)Cl]^+$ without prior aquation, and preliminary competition experiments were only performed at 297 K. These competition experiments between GSMe and 5′-GMP with $[Pt(dien)Cl]^+$ and $[Pt(dien)(H_2O)]^{2+}$ did not show intermolecular substitution of GSMe by 5′-GMP, and a study at higher temperature was felt desirable.

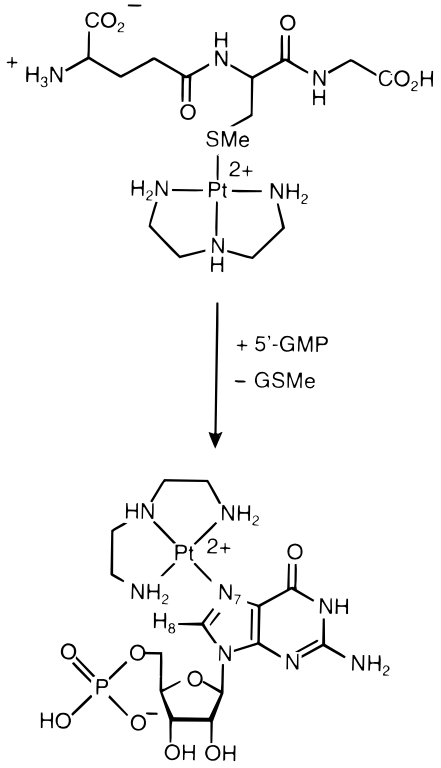
In this work the possibility of a ligand exchange reaction is addressed (e.g., for *cis*-$[Pt(NH_3)_2(GSMe)_2]^{2+}$ and $[P(dien)(GSMe)]^{2+}$ with GMP and d(GpG) (see Scheme 1). By varying the reaction time and temperature, the kinetics of the substitution reaction of $[Pt(dien)(GSMe)]^{2+}$ are also investigated.

Experimental Part

Chemicals. 5′-GMP, 5′-AMP, GSH, GSMe were obtained from Sigma Chemicals and used without further purification. d(GpG) was synthesized via the improved phosphotriester method.[16a]

Preparation of Pt Compounds. $[Pt(dien)Cl]Cl$ and cisplatin were prepared from K_2PtCl_4 according to literature procedures.[15b,16b] The identity of the Pt complexes was confirmed by infrared spectroscopy (Perkin-Elmer 580 spectrometer) and elemental analysis (Microanalytical Laboratory, University College, Dublin). $[Pt(dien)(GSMe)]Cl_2$ and *cis*-$[Pt(NH_3)_2(GSMe)_2]Cl_2$ were prepared in the NMR tube (D_2O was used as a solvent) by reaction of $[Pt(dien)Cl]Cl$ with 1 equiv of GSMe and of cisplatin with 2 equiv of GSMe at pH 3 and ambient temperature for 24 h. Reactions with cisplatin were performed in 4 mM NaCl solution. $[Pt(dien)(GS)]Cl$ was obtained by reaction in the NMR tube (D_2O as a solvent) of $[Pt(dien)Cl]Cl$ with 1 equiv of GSH at pH > 10 and ambient temperature for 15 min. Reaction in the NMR tube (D_2O as a solvent) of $[Pt(dien)Cl]Cl$ with 2 equiv of GSH at pH < 7 and ambient temperature for 24 h yielded $\{[Pt(dien)]_2(GS)\}Cl_3$. The presence of the desired compound was in each case confirmed by ^1H NMR spectroscopy. Any noncoordinated GSH/GSMe, or $[Pt(dien)Cl]^+$, could be reacted further by addition of one of the two components. After adjustment of the pH to 7 by DNO_3, the solutions were lyophilized. The resulting solids were kept at −20 °C and were used without further purification.

Scheme 1. Reaction of 5′-GMP with $[Pt(dien)(GSMe)]^{2+}$



pH Measurements. All pH measurements were performed at 298 K. The pH meter was calibrated with Fischer-certified buffer solutions of pH 4.00, 7.00, and 10.00. When D_2O was used as a solvent, meter readings were not corrected for deuterium isotope effects.

NMR Measurements; Experimental Conditions. The ^1H NMR and ^{195}Pt NMR measurements were recorded on Bruker WM 300 and DPX 300 spectrometers. D_2O was used as a solvent. For ^1H NMR, chemical shifts (δ) are calibrated to TMA at 3.18 ppm relative to TMS. ^{195}Pt spectra are referenced to K_2PtCl_6 (external reference). For monitoring the pH-dependent chemical shift behavior of the ^1H NMR signals, the pH was adjusted with 0.01−1 M solutions of NaOD or DCl.

Reactions Followed by ^1H NMR. Spectra were recorded at 295 K or 308 K. All reactions of platinated GSH and GSMe with 5′-GMP, 5′-AMP, and d(GpG) and the competition reactions between GSMe and 5′-GMP {$[Pt(dien)Cl]Cl$:GSMe:5′-GMP = 1:1:1} were carried out in the NMR tube (D_2O as a solvent) at 5 mM and 10 mM substrate concentrations and at several pH values. When the pH was 5 or 7, phosphate buffers (50 mM) were used. Concentrations of 5′-GMP (or 5′-AMP) were adjusted to abstract all platinum; this implies 2 equiv for $\{[Pt(dien)]_2(GS)\}^{3+}$ and 1 equiv for $[Pt(dien)(GS)]^+$ and $[Pt(dien)(GSMe)]^{2+}$. $[Pt(dien)(GSMe)]^{2+}$ and d(GpG) were reacted in a 1:1 ratio. The reaction of *cis*-$[Pt(NH_3)_2(GSMe)_2]^{2+}$ with GMP (1:2) was carried at 1 mM concentration in 10 mL of D_2O. For the ^{195}Pt NMR, this was

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lyophilized after 11 days and redissolved in 500 μL of D_2O to yield a 20-mM sample.

^{1}H NMR measurements were also used to obtain the rate constants and $t_{1/2}$ values of the reaction between 5′-GMP and [Pt(dien)(GSMe)]$^{2+}$. The reaction was carried out at pH 7 in 50 mM phosphate buffer with equimolar amounts (13.5×10^{-3} M initial concentrations) of [Pt(dien)-(GSMe)]$^{2+}$ and 5′-GMP. The temperature was 295 K, 301 K, 308 K, or 320 K. Calculations were performed by relative integration (estimated error is 5%) of H1′ proton signals of both reaction products and starting materials during the reaction. The values of the rate constants were determined from second-order Guggenheim plots.[17]

Results and Discussion

Ligand-Exchange Reactions. Reaction of GSMe and GSH with 1 or 2 equiv of [Pt(dien)Cl]Cl leads to formation of, respectively, [Pt(dien)(GSMe)]$^{2+}$, [Pt(dien)(GS)]$^{+}$, and {[Pt-(dien)]$_2$(GS)}$^{3+}$. Reacting 2 equiv of GSMe with cisplatin leads to the formation of *cis*-[Pt(NH$_3$)$_2$(GSMe)$_2$]$^{2+}$. Such complexes are well characterized,[18] and for all complexes coordination at the sulfur atom is observed without detectable side reactions. For *cis*-[Pt(NH$_3$)$_2$(GSMe)$_2$]$^{2+}$ this was confirmed with ^{195}Pt NMR.

Previous studies concerning the mechanism of action of rescue agents[15] showed that the complexes can be used as model adducts for platinum−protein binding (see Figure 1).

Figure 2 shows the course of the reaction between [Pt(dien)-(GSMe)]$^{2+}$ and 5′-GMP (1 equiv) when followed in time by ^{1}H NMR at 37 °C and pH 7. The H8 and H1′ signals of free 5′-GMP show a decrease in intensity, while simultaneously new H8 and H1′ signals arise. The chemical shift of the new H8 signal is 0.67 ppm downfield, compared with the H8 signal of free 5′-GMP, being characteristic for N7 coordination.[19] Further evidence for N7 coordination can be derived from the absence of a protonation effect at low pH[20] in the pH-dependent behavior of the H8 of the end product as is shown in detail in Figure 3. Moreover, the 5′-GMP proton signals of the end product are identical to those belonging to [Pt(dien)(5′-GMP)]$^{2+}$ formed by direct reaction of 5′-GMP and [Pt(dien)Cl]Cl.

Comparison of the GSMe proton signals generated during reaction with those of the starting complex [Pt(dien)(GSMe)]$^{2+}$ shows an upfield chemical shift of the protons closest to sulfur. The appearance of a singlet at 0.45 ppm upfield from the singlet belonging to the methyl protons of [Pt(dien)(GSMe)]$^{2+}$ unambiguously indicates release of GSMe during reaction; this release is confirmed by the fact that, at the end of the reaction, all GSMe proton signals are found at the position of free GSMe.[18] Further evidence for this assignment came from the addition of either GSMe or [PtCl(dien)]Cl at the end of the reaction. The proton signals of the added GSMe coincide with those of GSMe formed during reaction, whereas addition of another equivalent of [PtCl-(dien)]Cl leads to formation of [Pt(dien)(GSMe)]$^{2+}$.

The observed intermolecular rearrangement of Pt(dien)$^{2+}$ also occurs at ambient temperature, although the reaction proceeds considerably more slowly at that temperature. Use of 5′-AMP instead of 5′-GMP in the reaction with [Pt(dien)(GSMe)]$^{2+}$ did not show any significant GSMe release, which indicates that the rearrangement is indeed specific for guanine. Recently, a similar specificity for guanine was reported by Barnham et al.[21] in related studies on the substitution of the amino acid methionine in the preformed [Pt(dien)(Met-*S*)]$^{2+}$ adduct.

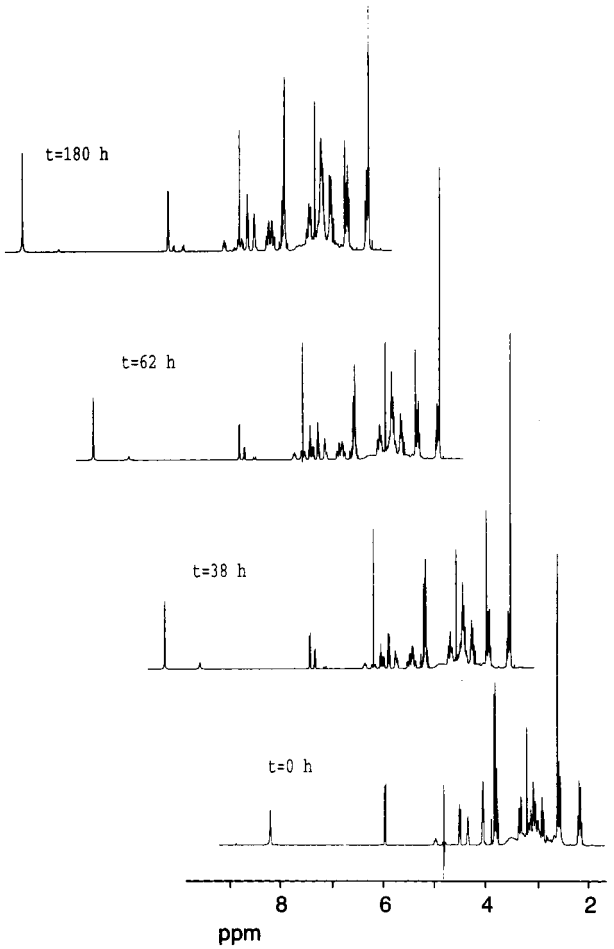

Figure 2. ^{1}H NMR spectrum of the reaction between [Pt(dien)(GSMe)]-Cl$_2$ (13 mM) and 1 equiv of 5′-GMP as a function of time (pH 7, T = 310 K). Chemical shifts are in ppm relative to DSS.

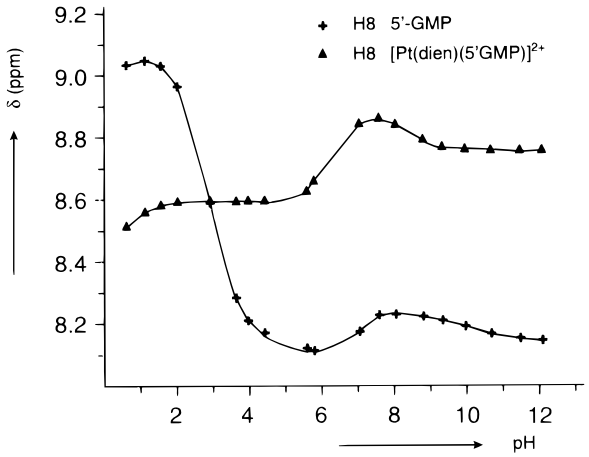

Figure 3. Chemical shifts (δ) of the H8 protons of free 5′-GMP and [Pt(dien)(5′-GMP)]$^{2+}$ as a function of pH at 295 K.

Competition Studies. Reacting [Pt(dien)(GSMe)]$^{2+}$ with d(GpG), a dinucleotide, to model the highly active GpG site in

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DNA also resulted in substitution of the sulfur on platinum by the N7 of the guanine, analogous to the reaction with 5′-GMP. The sulfur of the GSMe was found to be deplatinated at a similar rate as for the reaction with 5′-GMP. This was observed through the reappearance of the methyl signal of the free GSMe and appearance of new H8 signals in the aromatic region of the ^{1}H NMR spectrum. Platination of d(GpG) gave rise to two monoadducts [Pt(dien)(dGpG-5′N7)]$^+$ and [Pt(dien)(dGpG-3′N7)]$^+$ in about a 1:1 ratio, and only a small preference for the 5′G over the 3′G was observed. The bisadduct {[Pt(dien)]$_2$-(dGpG-5′N7,3′N7)}$^{3+}$ was not observed in detectable amounts.

To assess whether this intermolecular reaction also proceeds in the case of cisplatin, the cisplatin diadduct of GSMe, *cis*-[Pt(NH$_3$)$_2$(GSMe)$_2$]$^{2+}$, was reacted with 2 equiv of 5′-GMP at room temperature. Within 5 days a similar shift in the H8 proton signal could be observed as discussed above for the reaction with Pt(dien); a new peak appeared 0.5 ppm downfield from the original H8 signal, identical to the peak formed in the direct reaction of 5′-GMP with cisplatin. This peak can thus be assigned to the *cis*-[Pt(NH$_3$)$_2$GMP$_2$] complex. In addition to this peak, a smaller peak even more downfield (0.62 ppm downfield from free H8) could be observed, tentatively assigned to the monoGSMe, monoGMP adduct. A ^{195}Pt NMR spectrum recorded after 11 days of incubation confirmed the ligand substitution reaction on platinum. Three peaks were observed at −3390 ppm, −2911 ppm, and −2442 ppm. After comparing with literature values for similar reactions with GSH,[18] these signals could be assigned to *cis*-[Pt(NH$_3$)$_2$(GSMe)$_2$]$^{2+}$, *cis*-[Pt(NH$_3$)$_2$(GSMe)(GMP)]$^{2+}$, and *cis*-[Pt(NH$_3$)$_2$GMP$_2$]. The diadduct of 5′-GMP was clearly most abundant, showing that after 11 days most of the cisplatin-bound GSMe had been substituted by 5′-GMP.

In a separate experiment, the competition in Pt(dien)$^{2+}$ binding between GSMe and 5′-GMP was studied at 37 °C and pH 7. The reaction was followed with ^{1}H NMR {ratio used: [Pt(dien)Cl]Cl/GSMe/5′-GMP = 1:1:1}. Initially, the expected coordination at thioether sulfur was observed. During the course of the reaction, however, 5′-GMP substitutes coordinated GSMe, resulting in the [Pt(dien)(5′-GMP)]$^{2+}$ adduct as is depicted in Figure 4.

It appears that this S→N7 substitution is unique for thioethers, since addition of 5′-AMP or 5′-GMP to the cysteine model adducts [Pt(dien)(GS)]$^+$ and {[Pt(dien)]$_2$(GS)}$^{3+}$ at 37 °C does not show any detectable reaction. Because it is known that the stability of [Pt(dien)(GS)]$^+$ decreases at low pH[18] and that the pH of a tumor cell is slightly lower than the pH of a normal cell,[22] reactions with 5′-AMP and 5′-GMP were also performed at pH 5.00. Again, no GSH release was found.

Determination of Rate Constants. The values of k for the intermolecular rearrangement of Pt(dien)$^{2+}$ from GSMe to 5′-GMP were obtained from ^{1}H NMR measurements of the reaction mixture containing [Pt(dien)(GSMe)]$^{2+}$ and 5′-GMP in the ratio 1:1. The rate constants k were determined as in eq 1;[17] where x is the amount of [Pt(dien)(5′-GMP)]$^{2+}$ and a_0 is the initial concentration of [Pt(dien)(GSMe)]$^{2+}$.

$$k_2 t = x/[a_0(a_0 - x)] \quad (1)$$

A plot of the right side of eq 1 vs time shows a straight line passing through the origin as shown in Figure 5. The value of k was calculated from the slope of this line. The obtained kinetic data, given in Table 1, show that the reaction is a second-order process, which implies a direct attack of the N7 of 5′-GMP at [Pt(dien)(GSMe)]$^{2+}$.

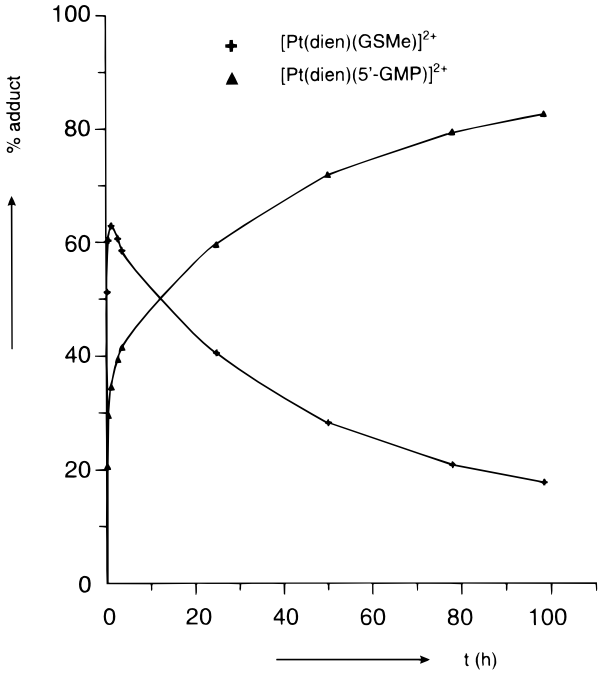

Figure 4. Observed product formation during the competition reaction [Pt(dien)Cl]Cl/5′-GMP/GSMe = 1:1:1.

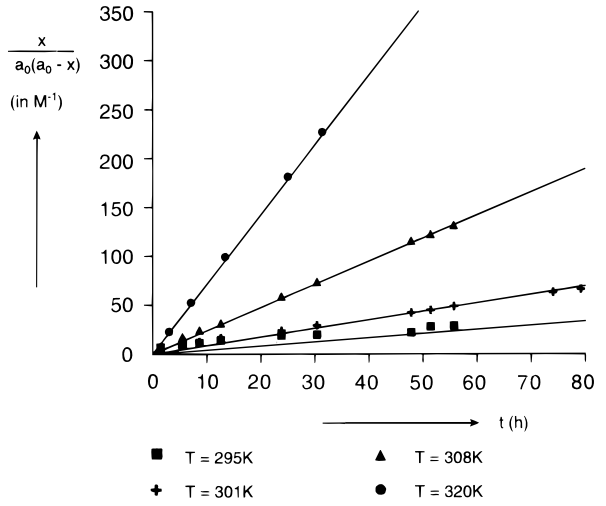

Figure 5. Second-order Guggenheim plots for the reaction of [Pt(dien)(GSMe)]Cl$_2$ with 5′-GMP at pH 7 and T = 295 K, 301 K, 308 K, or 320 K.

Table 1. Rate Constants (k in M^{-1}s^{-1}) and Half-Lives ($t_{1/2}$ in h) for the Reaction of [Pt(dien)(GSMe)]Cl$_2$ with 5′-GMP at pH 7 and T = 295 K, 301 K, 308 K, and 320 K

T (K)	k_2 (10^{-4} M^{-1} s^{-1})	$t_{1/2}$ (h)
295	1.15	179
301	2.41	85.4
308	6.56	31.4
320	197	10.4

Because the rate constants were determined at several temperatures, the $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ of the intermolecular substitution of GSMe by 5′-GMP could be deduced from an Arrhenius plot[23] using the data given in Table 1 (figure not shown). The values of 90(5) kJ mol^{-1} and −16(17) J K^{-1} mol^{-1} for, respectively,

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$\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ (at 295 K) are in agreement with an associative mechanism of ligand substitution.[23]

Mechanistic Considerations. The experiments on *S*-guanosyl-L-homocysteine described before[10] proved for the first time that the N7 of guanine can replace a sulfur donor atom in a platinum−thioether adduct. This intramolecular substitution reaction proved to be specific for guanine. Subsequent competition experiments used a nucleopeptide model system, Met−TpG,[12] and have confirmed this ligand substitution. The results on the Pt−cysteine and Pt−methionine model adducts presented in this paper demonstrate that GSMe can be selectively released from $[Pt(dien)(GSMe)]^{2+}$ by G−N7 in 5′-GMP and d(GpG) and even in the case of the cisplatin−GSMe adduct $[Pt(NH_3)_2(GSMe)_2]^{2+}$. These results show that the substitution by 5′-GMP is limited to platinum−sulfur adducts of the methionine type; platinum−cysteine model adducts proved to be stable under these conditions. The findings described in this paper also show that the replacement of a sulfur donor atom in a thioether−platinum adduct is not limited to an intramolecular rearrangement, which is in agreement with the report of intermolecular substitution of the amino acid methionine.[21]

The observation that 5′-GMP cannot release GS^- at 37 °C from the $[Pt(dien)(GS)]^+$ adduct is consistent with results of experiments where rescue agents were used to reverse platinum−sulfur binding in the same model adducts. These results show that even strong nucleophiles such as ddtc, thiourea, and WR-2721 cannot reverse the Pt-cysteine bond.[15]

Interestingly, $\{[Pt(dien)]_2(GS)\}^{3+}$, which in a way can be compared with a Pt−thioether adduct, also does not react with 5′-GMP at 37 °C. Reactions with several rescue agents[15] on this system did show the formation of $[Pt(dien)(GS)]^+$. The reactivity of $\{[Pt(dien)]_2(GS)\}^{3+}$ in that case, however, was found to be at least 10 times lower than that of the actual Pt−thioether adduct $[Pt(dien)(GSMe)]^{2+}$. Because the overall reactivity of G−N7 toward platinum−sulfur adducts is considerably lower than that of rescue agents, the observed stability of $\{[Pt(dien)]_2(GS)\}^{3+}$ in the presence of 5′-GMP is not surprising.

The substitution by 5′-GMP in $[Pt(dien)(GSMe)]^{2+}$ adducts is rather slow at ambient temperature ($t_{1/2} = 179$ h). This may explain why this reaction was not considered important in previous experiments.[15b] At 35 °C, however, the half-life is 31 h. Because part of the administered cisplatin is not excreted from the body within 24 h,[24] the observed release of thioether sulfur at 37 °C is also relevant for in vivo processes. In the present paper it has been shown that the results obtained with the adducts of monofunctional [Pt(dien)Cl]Cl can also be extrapolated to cisplatin, as the intermolecular substitution of the sulfur atom by the N7 atom was also proven to take place with adducts of cisplatin. The reaction takes place on a similar time scale for both complexes, but the exact kinetics of these substitution reactions have as yet only been determined for [Pt(dien)Cl]Cl, and different kinetics can be expected for cisplatin.

The intermolecular rearrangement described in this paper may have important biological implications because it supports the hypothesis of a drug reservoir mechanism[8] in which the platinum−sulfur adduct is an intermediate for platinum binding at DNA. In vivo, such a mechanism could operate under circumstances that reduce the role of the aquation pathway (high Cl^- concentration) usually found for cisplatin and that accelerate the substitution process. The latter accelerating circumstances include high concentration (e.g., in the nucleus) and favorable orientation of the reactants. A favorable orientation is likely to occur when proteins are located in close vicinity of, or even bind to, DNA. Histones,[25] with their attachment to DNA, are a typical example of such proteins. With DNA-binding proteins the substitution by the N7 of guanine might be accelerated considerably. In fact, this substitution process (Figure 6A) almost resembles an *intra*molecular rearrangement, as modeled by the previously reported SGH[10] and the nucleopeptide Met-TpG,[12] with different distances between guanine−N7 and thioether sulfur. A second possible structure in which rearrangement might occur is depicted in Figure 6B. These cisplatin cross-links[26] between a protein and DNA are known to occur in vivo. In this case the platinum−sulfur adduct could react with the N7 of the guanine adjacent to the guanine that is already coordinated, eventually forming a GpG adduct. This process also resembles an intramolecular mechanism, so it could proceed faster than the intermolecular rearrangements described here.

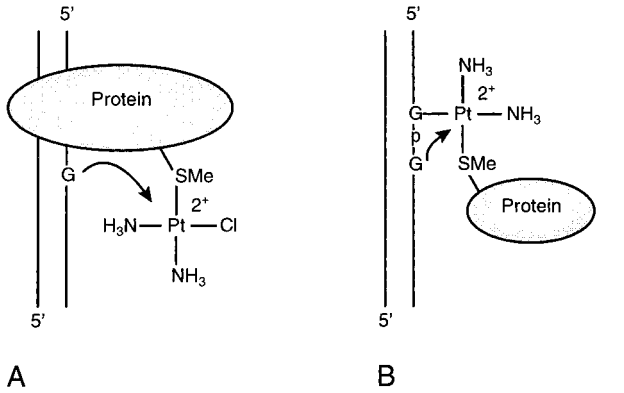

Figure 6. Possible structures with a favorable orientation of the thioether function and the N7 of guanine in (*A*) DNA-binding proteins and (*B*) the cis-platinum cross-link between DNA and a protein.

The results presented here strongly indicate that the rearrangements described above are limited to the platinum−sulfur adduct of the methionine type. Platinum−sulfur adducts of the cysteine type, like the adducts formed with glutathione, are shown to be unreactive toward the N7 of guanine. These adducts are therefore unlikely candidates for a drug reservoir.

Another, related hypothesis supported by the intermolecular rearrangement is the activation of platinum compounds with slowly hydrolyzing leaving groups such as CBDCA[9] and aminophosphonic acid.[27] With these leaving groups, 5′-GMP was found to react by direct nucleophilic attack without prior aquation. This process might be accelerated by reaction with sulfur-containing nucleophiles as an intermediate step. Again, the above-cited results indicate that such activation is limited to thioether sulfur atoms.

A third consequence is derived from the observed specificity for guanine and not adenine. This selective behavior was also found for the intramolecular rearrangement and could be an additional explanation for the observed preference for guanine platination in vivo.[28]

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Concluding Remarks

In conclusion, it is shown that only the sulfur donor atom in a platinum−sulfur adduct of the thioether type can be selectively substituted by G−N7; the platinum−cysteine model adducts appear to be too stable for purine−N7 substitution.

The results of these intermolecular substitution experiments on model adducts for platinum−sulfur adducts in proteins add significant evidence to the idea of a drug reservoir of platinum−thioether adducts existing in vivo. Another implication concerns the possibility of activation by thioether sulfur of platinum compounds with slowly hydrolyzing leaving groups. In addition, the confirmation of selectivity for guanine in the substitution process could form an additional explanation for the observed preference for guanine platination in vivo. It should be noted, however, that these results are obtained from model adducts of platinum protein adducts; in vivo conditions can still be different.

Finally, the possibility of replacing a thioether sulfur atom by the N7 of guanine in a platinum−sulfur adduct could eventually result in the development of a new class of platinum antitumor compounds in which the thioether function is already incorporated as the leaving ligand. These new compounds would have completely different kinetic properties. Because the observed negative side effects of cisplatin, such as nephrotoxicity, inactivation, and resistance, appear to be closely related to its kinetics,[29] the new platinum compounds could have a lower toxicity while still being able to react with guanine−N7 coordination sites of DNA. Current research is dealing with this new class of platinum compounds.

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