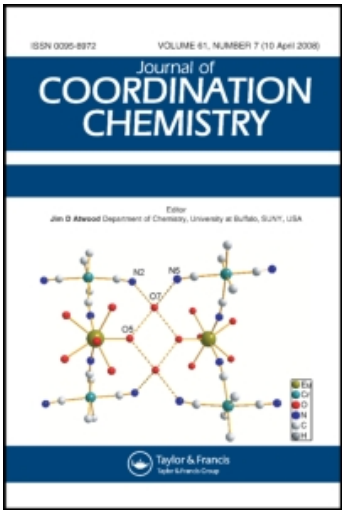

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^{1}H NMR INVESTIGATION OF COMPETITIVE BINDING OF SULFUR-CONTAINING PEPTIDES AND GUANOSINE 5'-MONOPHOSPHATE TO A MONOFUNCTIONAL PLATINUM(II) COMPLEX

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^{1}H NMR INVESTIGATION OF COMPETITIVE BINDING OF SULFUR-CONTAINING PEPTIDES AND GUANOSINE 5′-MONOPHOSPHATE TO A MONOFUNCTIONAL PLATINUM(II) COMPLEX

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The competitive binding of *N*-acetylglycyl-D,L-methionine (AcGly-Met) and guanosine-5′-monophosphate (5′-GMP) to the monofunctional $[Pt(dien)Cl]^+$ complex has been studied by ^{1}H NMR spectroscopy. Results have shown that in the initial stages of the reaction the platinum(II) complex only reacts with the methionine containing peptide, and the second step of this reaction is the very slow intermolecular displacement of the *S*-bound thioether ligand with the *N*7 atom of the guanine base of 5′-GMP. Comparative reactions of glutathione have also been investigated. The obtained results have been analyzed in relation to the antitumour activity and toxicity of platinum complexes.

Keywords: Platinum(II); peptide; guanosine-5′-monophosphate; proton NMR; intermolecular displacement

INTRODUCTION

In recent years, there has been an increasing interest in the interactions between the platinum drugs and sulfur-containing biomolecules.[1] These interactions are thought to be responsible for a variety of biological effects,

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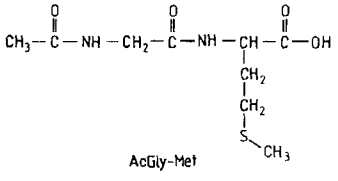
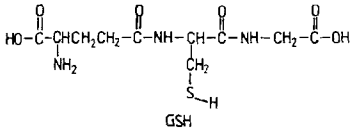
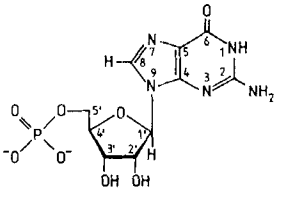
such as inactivation of Pt(II) complexes, development of cellular resistance to platinum, and toxic side effects such as nephrotoxicity.[2] The thioether-containing amino acid methionine plays an important role in the metabolism of platinum anticancer drugs. Recent results obtained by NMR investigations of the kinetics and thermodynamics of the competitive binding of L-methionine (L-Met) and guanosine-5′-monophosphate (5′-GMP) to the monofunctional $[Pt(dien)Cl]^+$ complex (dien = 1,5-diamino-3-azapentane) in aqueous solution have shown that 5′-GMP selectively displaces Pt-bound L-Met.[3] This, together with the results of van Boom and Reedijk,[4] who reported the intermolecular displacement of a Pt-bound thioether by a guanine nucleobase, suggest that novel routes to DNA platination by anticancer drugs may exist. It has also been found that the reaction of 5′-GMP with cisplatin, *cis*-$[PtCl_2(NH_3)_2]$, is faster in the presence of L-methionine than in its absence.[5] Recent results of Barnham *et al.*[6] have also confirmed that slow intermolecular displacement of *S*-bound L-Met ligand by the *N*7 nitrogen atom of guanine occurs in the reactions of two platinum(II) complexes, $[Pt(en)(MeCO\text{-}Met\text{-}S)Cl]^+$ and $[Pt(en)(MeCO\text{-}Met\text{-}S)_2]^{2+}$ (en = ethane-1,2-diamine, MeCO-Met = *N*-acetyl-L-methionine), with 5′-GMP and GpG [guanylyl(3′-5′)guanosine] oligonucleotides.







SCHEME I

To investigate this possibility further we have studied the reaction of the monofunctional [Pt(dien)Cl]Cl complex with peptides *N*-acetylglycyl-*D,L*-methionine (AcGly-Met) and glutathione (GSH) in the presence mononucleotide guanosine-5′-monophosphate (5′-GMP). Elucidation of the reaction pathway was aided by the use of ^{1}H NMR spectroscopy in phosphate buffer at pD = 7.4.

EXPERIMENTAL

Materials

Distilled water was demineralized and purified to a resistance greater than 10 MΩ cm. D_2O and $K_2[PtCl_4]$ were obtained from Aldrich Chemical Co. Glycyl-*D,L*-methionine (Gly-Met), glutathione (GSH) and disodium guanosine-5′-monophosphate (5′-GMP) were purchased from Sigma Chemical Co. All other chemicals were of reagent grade. The terminal amino group in Gly-Met was acetylated by a standard procedure.[7,8] [Pt(dien)Cl]Cl was synthesized by a published procedure.[9] The purity of the complex was checked by elemental microanalyses and ^{1}H NMR spectroscopy.

pH Measurements

All pH measurements were performed at 298 K. The pH meter (Iskra MA 5704) was calibrated with Fischer certified buffer solutions at pH 4.00 and 7.00. The pH values were converted to pD by the standard formula[10] pD = pH+0.41. However, in conceptual reference to acidity and basicity the common symbol pH is used.

^{1}H NMR Measurements

Proton NMR spectra were recorded with Varian Gemini 200 and Bruker 250 spectrometers. The reaction of $[Pt(dien)Cl]^+$ with AcGly-Met and 5′-GMP was followed by ^{1}H NMR spectroscopy. Equimolar amounts of the platinum(II) complex, AcGly-Met and 5′-GMP were mixed in an NMR tube. The final solution was 10 mM in each reactant. All reactions were carried out at room temperature in 50 mM phosphate buffer at pD 7.4 in D_2O as solvent. The internal reference was TSP (sodium trimethylsilylpropane-3-sulfonate).

RESULTS AND DISCUSSION

The reaction of $[Pt(dien)Cl]^+$, **1**, with *N*-acetylglycyl-D,L-methionine (AcGly-Met) and guanosine-5′-monophosphate (5′-GMP) was carried out at room temperature in 50 mM phosphate buffer at pD 7.4. The formation of products in this reaction (Figure 1) was monitored by ^1H NMR spectroscopy using the *S*-methyl protons of AcGly-Met and the H8 proton of 5′-GMP. The SCH_3 signal of free AcGly-Met was observed at 2.13 ppm and for the *S*-bound peptide this signal was shifted downfield by 0.41 ppm (δ 2.54 ppm). The chemical shifts of the H8 proton for the free and for *N*7 coordinated 5′-GMP are at 8.17 and 8.85 ppm, respectively. In the initial stages of the reaction the SCH_3 signal for free AcGly-Met (2.13 ppm) decreased in intensity and a new signal characteristic of $[Pt(dien)(AcGly\text{-}Met\text{-}S)]^+$, **2**, appeared in the spectrum, whereas little of the 5′-GMP reacted (Figure 2). In the later stages, the signals for bound AcGly-Met and free 5′-GMP decreased in intensity, whereas those for free AcGly-Met increased in intensity, as did those assignable to bound 5′-GMP in [Pt(dien)(5′-GMP-*N*7)], **3** (Figure 2).

Time dependence of the concentration of AcGly-Met in the reaction with complex **1** in the presence of 5′-GMP is shown at Figure 3. In a separate experiment with complex **1** and AcGly-Met in 50 mM phosphate buffer at pD 7.4, after 10 days no increase of the peak for SCH_3 protons of the free peptide was observed. From these results it is notable that $[Pt(dien)(AcGly\text{-}Met\text{-}S)]^+$ represents kinetically preferred initial product while the [Pt(dien)(5′-GMP-*N*7)] is much more thermodynamically stable.

For comparison, similar work has been carried out by using glutathione (GSH) instead of AcGly-Met. First we prepared the $[Pt(dien)GS]^+$ complex by mixing equimolar amounts of **1** with GSH in D_2O at pH > 10. This reaction mixture was left for 15 mins at room temperature, and then the pH of the solution was reduced to *ca* 7 by using DNO_3. The $[Pt(dien)GS]^+$ complex was then reacted with an equivalent amount of 5′-GMP and the reaction followed by ^1H NMR spectroscopy. No changes in the ^1H NMR spectrum were observed over a three-week period. This result, along with others,[2-6,9,11] suggests that Pt-thiolate complexes are very stable and that intermolecular displacement of *S*-bound deprotonated thiolate ligands, such as glutathione, by the *N*7 atom of 5′-GMP is not possible.

In separate experiments we confirmed that the reaction of **1** with AcGly-Met alone is relatively fast, and that complex **3** can be formed from **2** by direct displacement of coordinated AcGly-Met by 5′-GMP. The rate of reaction of Gly-Met with **1** is similar to that reported previously for L-Met

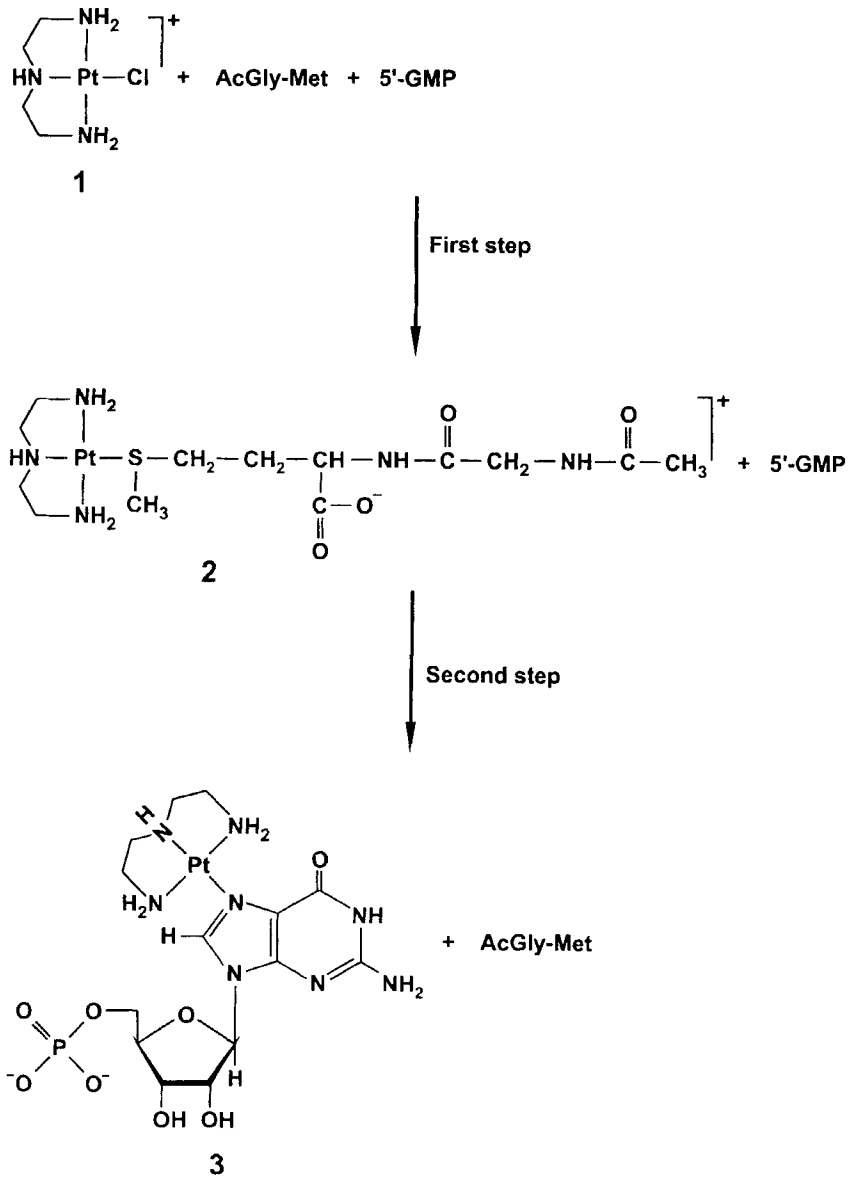


FIGURE 1 A possible scheme for the competitive binding of AcGly-Met and 5′-GMP to [Pt(dien)Cl]Cl at pD 7.4 in 50 mM phosphate buffer.

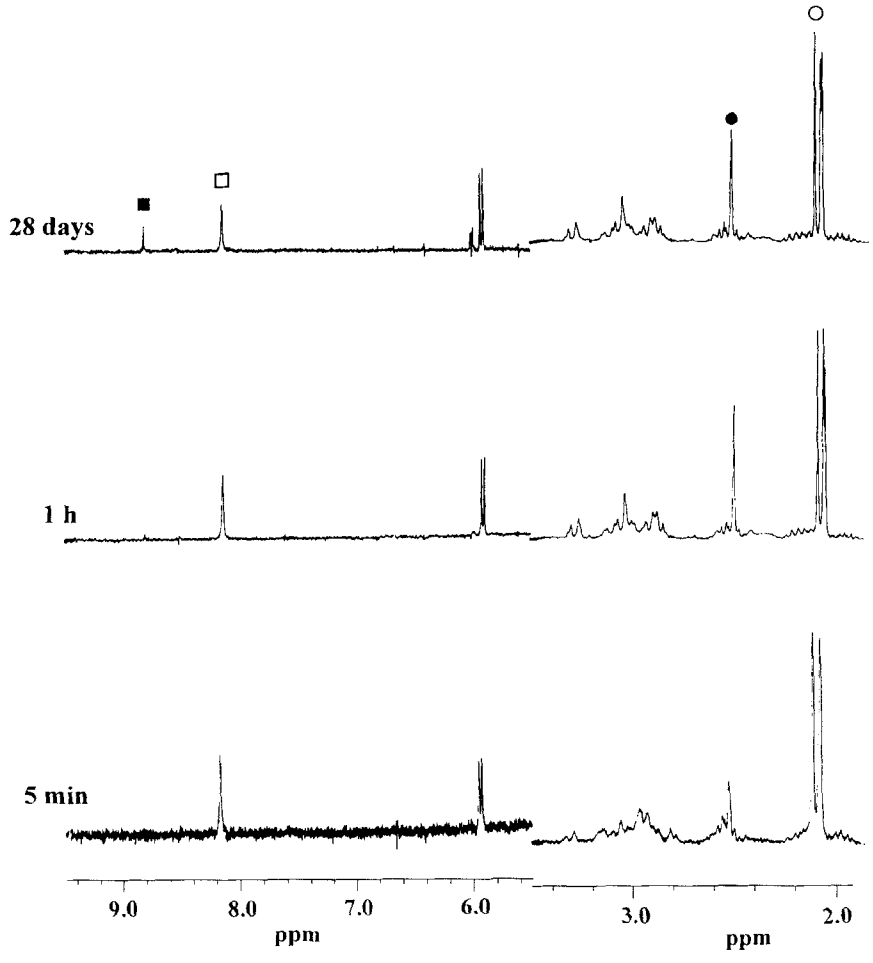


FIGURE 2 Parts of the ^{1}H NMR spectra for the reaction of $[Pt(dien)Cl]^+$ with AcGly-Met in the presence of 5′-GMP (1 : 1 : 1 mol ratio) as a function of time at pD 7.4 in 50 mM phosphate buffer and D_2O as solvent. Chemical shifts are in ppm relative to TSP. Resonances are indicated as follows: bound (■) and free (□) 5′-GMP; bound (●) and free (○) AcGly-Met.

and *S*-methylglutathione, GSMe (see Table I). The peptide Gly-Met and complex **1** were mixed in a 1 : 1 mol ratio in D_2O at pD 3.50. The reaction was followed by observing the decrease in intensity of the ^{1}H NMR signal of SCH_3 for the free peptide (δ 2.13 ppm) and increase in intensity of the signal at 2.54 ppm for **2**. The value of the rate constant was determined when the data from the early part of the reaction (up to 2 h) were fitted to a

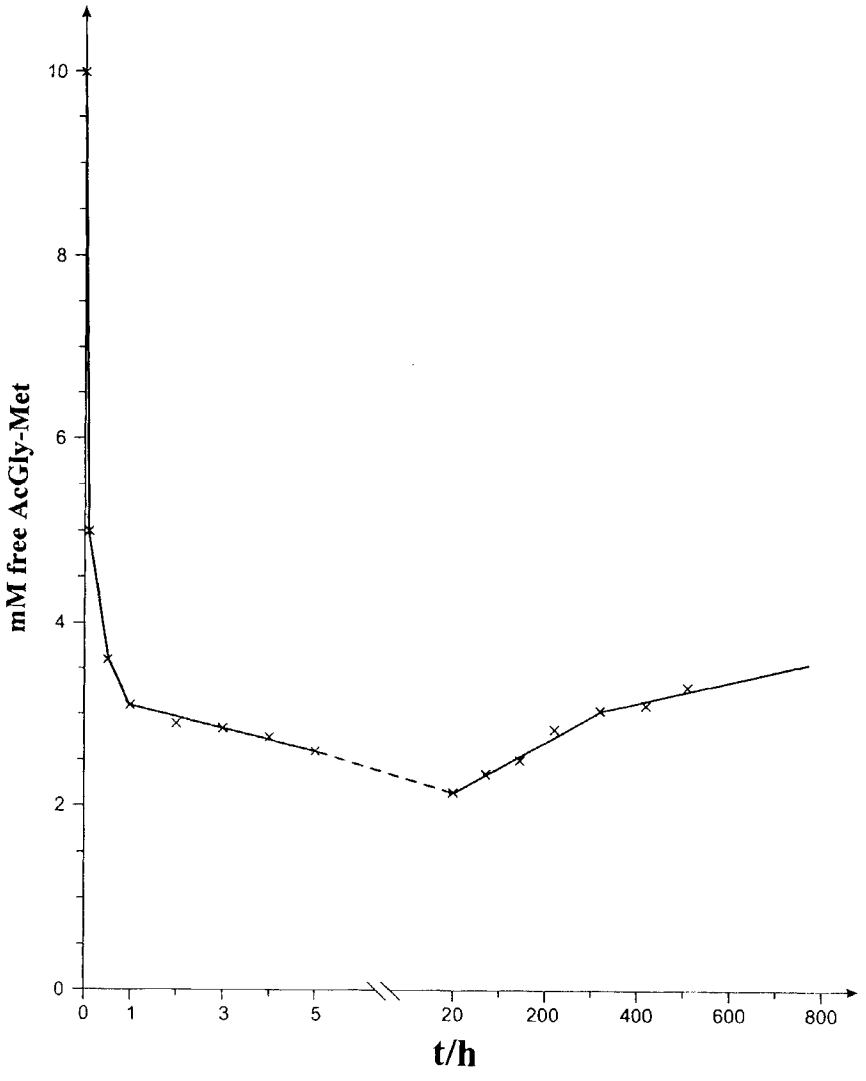


FIGURE 3 Time dependence of the concentration of AcGly-Met in the reaction with $[Pt(dien)Cl]^+$ in the presence of 5′-GMP (1 : 1 : 1 mol ratio).

second-order process[12] by plotting $x/a_0(a_0 - x)$ *vs* t (a_0 = initial concentration of Gly-Met and x = concentration of $[Pt(dien)(Gly\text{-}Met\text{-}S)]^+$ at time t), yielding a rate constant of 44×10^{-3} $M^{-1}s^{-1}$ (Table I).

These findings have implications for the mechanism of action of platinum anticancer drugs. Sulfur ligands are generally thought to have a much higher affinity for platinum(II) than nitrogen ligands and to diminish the

TABLE I Kinetic data for the reaction of $[Pt(dien)Cl]^+$ with thioether-containing ligands, where k_2 is the second-order rate constant

Ligand	*pD*	T/K	$k_2 \times 10^{-3}$ dm^3 mol^{-1} s^{-1}	*Ref.*
L-Met	4.31	300	14	3
GSMe[a]	5.41	295	33	9
Gly-Met	3.50	295	44	this work

[a] GSMe = *S*-methylglutathione.

antitumour activity of platinum complexes.[13] Our results are in agreement with those obtained for the reaction of L-Met with $[Pt(den)Cl]^+$ and *cis*-$[PtCl_2(NH_3)_2]$ in the presence of 5′-GMP.[3,5] Moreover, we show that intermolecular displacement reactions may be facilitated if the methionine adduct is formed not simply by Met itself, than by an accessible Met residue on a methionine-containing peptides and proteins. This observation could have important consequences, because it supports the hypothesis of a drug reservoir mechanism in which Pt (initially) bound to a protein may react further to yield Pt bound to DNA. The GMP-Met displacement reactions are very slow, but it is notable that a very slowly excreted pool of Pt exists *in vivo* after administration of cisplatin (which has a half-life of several days).[14]

^{1}H NMR investigations of the competitive binding of glutathione and guanosine 5′-monophosphate to $[Pt(dien)Cl]^+$ have shown that $[Pt(dien)GS]^+$ is unreactive towards 5′-GMP. Although thiols, such as glutathione, are also abundant *S*-containing ligands in cells, we have shown that Pt initially bound to a deprotonated thiol group can not react further with DNA. The latter finding, together with numerous previous results,[2,9,11,15-17] clearly confirms that sulfur-containing biomolecules including amino acids such as cysteine, peptides such as glutathione, and proteins such as metallothionein, and many others, are responsible for inactivation of Pt antitumour complexes, development of cellular resistance to platinum, and toxic side effects such as nephrotoxicity.

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