L-Methionine increases the Rate of Reaction of 5'-Guanosine Monophosphate with the Anticancer Drug Cisplatin: Mixed-ligand Adducts and Reversible Methionine Binding†

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L-Methionine (L-HMet) increased the rate of reaction of the anticancer drug cisplatin, *cis*-$[PtCl_2(NH_3)_2]$, with guanosine 5'-monophosphate (5'-GMP) at pH 7. The course of the reaction has been elucidated by ^1H and [^1H, ^{15}N] NMR spectroscopy. Novel intermediates detected and characterized include *cis*-$[Pt(5'\text{-GMP-}N^7)(L\text{-HMet-}S)(NH_3)_2]^{2+}$ and $[Pt(L\text{-Met-}S,N)(5'\text{-GMP-}N^7)(NH_3)]^+$ (charges on 5'-GMP ignored), the formation of which involves ammine release. Monodentate S-bound L-HMet can co-ordinate reversibly, whereas S,N-chelated L-Met is much less reactive. Thus methionine residues in peptides and proteins could play a role in the transfer of Pt onto DNA. Comparative reactions of $[Pt(en)Cl_2]$ (en = 1,2-diaminoethane) have also been investigated.

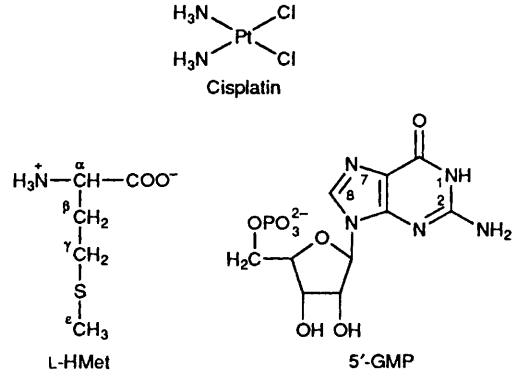
It is now widely accepted that the antitumour activity of platinum anticancer drugs is due to the platination of DNA, most commonly the guanine bases.[1,2] Recently interest in the interactions of Pt drugs with sulfur-containing biomolecules has grown markedly since they are thought to play a role in the severe nephrotoxicity of cisplatin, inactivation of Pt complexes and tumour resistance to treatment with Pt drugs.[3]

One such sulfur-containing biomolecule is L-methionine (L-HMet). The complex $[Pt(L\text{-Met-}S,N)_2]$ has been isolated from the urine of patients treated with cisplatin,[4] and recently an adduct similar to *cis*-$(Pt(cbdca\text{-}O)(NH_3)_2(L\text{-HMet-}S)]$ has been detected in the urine of mice treated with carboplatin $[Pt(cbdca\text{-}O,O')(NH_3)_2]$ (cbdca = cyclobutane-1,1-dicarboxylic acid).[5] These results indicate a potentially important role for L-HMet in the metabolism of Pt anticancer drugs.

The reactions of L-HMet with cisplatin and its hydrolysis product *cis*-$[Pt(NH_3)_2(H_2O)_2][NO_3]_2$ have been well documented.[6-8] The major products, $[Pt(L\text{-Met-}S,N)_2]$ and $[Pt(L\text{-Met-}S,N)(NH_3)_2]$, contain S,N-chelated L-Met, although at low pH (< 2) Appleton *et al.*[6] have identified an S,O-chelate as an additional product. Recent work in this laboratory,[9] with the complex $[Pt(dien)Cl]^+$ (dien = 1,5-diamino-3-azapentane), has shown that S-bound L-HMet in the adduct $[Pt(dien)(L\text{-HMet-}S)]^{2+}$ can be replaced by guanosine 5'-monophosphate (5'-GMP). This, together with the results of van Boom and Reedijk,[10] who reported the intramolecular displacement of a Pt-bound thioether by a guanine nucleobase, suggest that novel routes to DNA platination by anticancer drugs may exist. To investigate this possibility further we have studied the reaction of cisplatin with 5'-GMP in the presence of L-HMet.‡ Elucidation of the reaction pathway is aided by the use of *cis*-$[PtCl_2(^{15}NH_3)_2]$ **1** and [^1H, ^{15}N] heteronuclear multiple quantum coherence (HMQC) NMR spectroscopy, in addition to direct NMR detection of ^1H and ^{15}N nuclei.

† *Non-SI unit employed:* M = mol dm^{-3}.

‡ The charge on GMP is ignored in formulae.



Experimental

Materials.—L-Methionine (L-HMet) and disodium guanosine 5'-monophosphate were purchased from Sigma, and $[^{15}NH_4]_2SO_4$ (> 98% ^{15}N) from Aldrich. The complexes *cis*-$[PtCl_2(^{15}NH_3)_2]$ **1** and *cis*-$[Pt(^{15}NH_3)_2(H_2O)_2][NO_3]_2$ were prepared using standard methods,[11] while $[Pt([^{15}N]en)Cl_2]$ and $[Pt([^{15}N]en)(H_2O)_2][NO_3]_2$ (en = 1,2-diaminoethane) were prepared as previously described.[12]

Preparation of NMR Samples.—All samples for [^1H, ^{15}N] NMR spectroscopy were prepared in 90% H_2O–10% D_2O solutions.

cis-$[PtCl(L\text{-HMet-}S)(^{15}NH_3)_2]^+$ **2**. This was prepared by adding 0.25 ml of a 20 mM solution of L-HMet (5 μmol) to 0.25 ml of a 20 mM solution of *cis*-$[PtCl(^{15}NH_3)_2(H_2O)]^+$. The 20 mM solution of *cis*-$[PtCl(^{15}NH_3)_2(H_2O)]^+$ was prepared by slow addition, with stirring, of 1 mol equivalent of solid NaCl to a 20 mM solution of *cis*-$[Pt(^{15}NH_3)_2(H_2O)_2][NO_3]_2$. The NMR spectra of the product were recorded.

cis-$[Pt(5'\text{-GMP-}N^7)(L\text{-HMet-}S)(^{15}NH_3)_2]^{2+}$ **8**. A 1 ml aliquot of a 40 mM solution of 5'-GMP (pH adjusted to 4) was added to 1 ml of a 40 mM solution of *cis*-$[Pt(^{15}NH_3)_2$-

$(H_2O)_2][NO_3]_2$. This gave a solution containing predominately *cis*-$[Pt(5'\text{-GMP-}N^7)(^{15}NH_3)_2(H_2O)]^{2+}$. To a 0.25 ml aliquot of this solution was added 0.25 ml of a 20 mM solution of L-HMet, and NMR spectra of this were recorded.

$[Pt(\text{L-Met-}S,N)(5'\text{-GMP-}N^7)(^{15}NH_3)]^+$ **9**. To a 1 ml aliquot of a 40 mM solution of L-HMet was added 1 ml of a 40 mM solution of *cis*-$[Pt(^{15}NH_3)_2(H_2O)_2][NO_3]_2$. This was allowed to react for 10 min to form $[Pt(\text{L-Met-}S,N)(^{15}NH_3)_2]^+$.[6] To 0.25 ml of this solution was added 0.25 ml of a 20 mM 5'-GMP solution, and after standing for 20 min, NMR spectra were recorded.

$[Pt([^{15}N]en)(\text{L-Met-}S,N)]^+$ **10**. To 1 ml of a 20 mM solution of L-HMet was added 1 ml of 20 mM $[Pt([^{15}N]en)(H_2O)_2][NO_3]_2$. This solution was allowed to stand for 10 min before recording NMR spectra.

Reaction of cis-$[PtCl_2(^{15}NH_3)_2]$ *and* $[Pt([^{15}N]en)Cl_2]$ *with* 5'-GMP *and* L-HMet.—Reactions were performed on an NMR scale at 37 °C, *i.e.* 10 mM concentrations of reactants, 1 : 1 : 1 mol ratios, final volume 0.5 ml. When ^1H spectra were required, D_2O was the solvent. When ^{15}N and [^1H, ^{15}N] spectra were required, 90% H_2O–10% D_2O was used as the solvent. Reactions were preformed with and without the presence of 50 mM phosphate buffer (pH* = 7). All reactions were performed at 37 °C.

pH Measurements.—These were made directly in NMR tubes before and after recording spectra using a Corning 240 metre equipped with an Aldrich micro combination electrode, calibrated with Aldrich buffer solutions at pH 4, 7 and 10. Meter readings for D_2O solutions are termed pH*.

NMR Spectroscopy.—The 500.13 MHz ^{15}N-edited ^1H-{^{15}N} NMR and two-dimensional [^1H, ^{15}N] HMQC NMR spectra were recorded on a Bruker AM-500 spectrometer as previously described.[12,13]

Both one-dimensional ^{15}N-edited ^1H spectra and two-dimensional [^1H, ^{15}N] spectra were recorded using an HMQC sequence, optimised for $^1J(N\text{–}H) = 71$ Hz. Water suppression was achieved by presaturation for 1.5 s. Typically 128 transients were acquired in *ca.* 10 min. The ^{15}N spins were decoupled by irradiating with the GARP sequence at a field strength of 1.0 KHz. Two-dimensional spectra were processed using sine-bell weightings in both dimensions. The 40.1 MHz ^{15}N NMR spectra were recorded using direct detection on a Bruker AM-400 spectrometer, and 500.1 MHz ^1H NMR were recorded on a JEOL GSX500 spectrometer.

Proton NMR spectra were referenced to internal sodium 3-(trimethylsilyl)tetradeuteriopropionate (500 MHz spectra), and ^{15}N spectra to external 1.5 M NH_4Cl in 1 M HCl (90% H_2O–10% D_2O).

Results and Discussion

Reactions of cisplatin with L-HMet *and* 5'-GMP. *Proton and* ^{15}N *NMR.*—The reaction of cisplatin **1**, L-HMet and 5'-GMP in a 1 : 1 : 1 mol ratio at pH 7, 37 °C was initially followed by ^1H NMR spectroscopy. Although the reaction is complicated and many products are formed, the peaks due to H^8 of 5'-GMP and the S-methyl group of L-HMet provide useful probes for following the reaction (but not necessarily for characterization).

The ^1H NMR spectrum shows that the initial phase is dominated by the reaction of cisplatin **1** with L-HMet. The first change to occur in the ^1H NMR spectrum, observable within 10 min of mixing, is the growth of a singlet at δ 2.48. This peak is assignable to the S-methyl protons of L-HMet co-ordinated to platinum through the sulfur atom. It was present in the spectrum for up to 24 h of reaction time, but was never the major SMe species. A small peak was also observed at δ 2.63 but this overlapped with the triplet for the L-HMet γ-CH_2 group, and it was not possible to monitor its time dependence. The

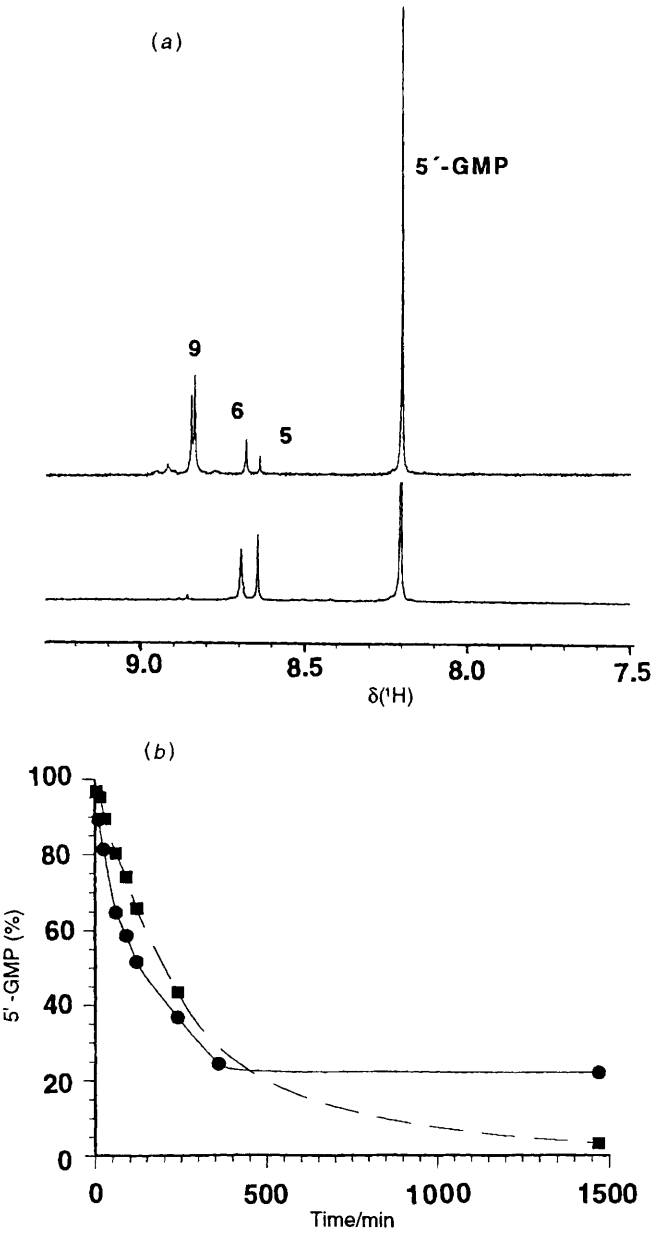


Fig. 1 (*a*) The 500 MHz ^1H NMR spectra of reaction mixtures containing cisplatin–5'-GMP (1 : 1), 37 °C, 2 h (bottom), and cisplatin–5'-GMP–L-HMet (1 : 1 : 1), 37 °C, 2 h, pH 7 (top). For assignments see Table 1. The formation of mixed-ligand complexes is notable in the presence of L-HMet. (*b*) Plot of intensities of the H^8 peak for 5'-GMP in the absence (■) and presence (●) of L-HMet. The increased rate of reaction in the early stages of the latter reaction is evident

appearance of these peaks was followed by two other peaks at δ 2.53 and 2.55, which have previously been assigned to the S-methyl groups of the diastereoisomers of $[Pt(\text{L-Met-}S,N)(NH_3)_2]^+$ **3**,[6,7] for which there is slow inversion of the chiral co-ordinated sulfur. Several singlets which are assignable to the S-methyl signals of the diastereoisomers of *cis*- and *trans*-$[Pt(\text{L-Met-}S,N)_2]$ **4** were also observed between δ 2.59 and 2.60.[8] As the reaction proceeds, further new peaks due to different S-methyl groups appear, mostly in the region δ 2.60–2.70. The overlap of these peaks with the γ-CH_2 of L-HMet makes quantitation difficult.

Surprisingly the rate of disappearance of 5'-GMP in the initial stages of the reaction (first 2 h) appeared to be about twice that observed for the reaction of cisplatin with 5'-GMP alone under similar conditions. These data obeyed approximate first-order kinetics (Fig. 1), from which pseudo-first order rate

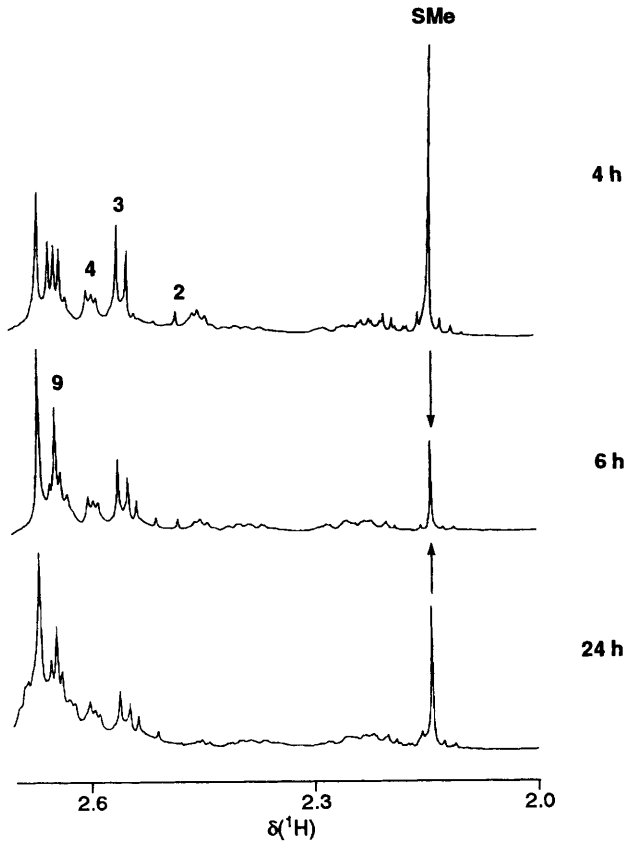


Fig. 2 The 500 MHz ^{1}H NMR spectra of a solution containing cisplatin, 5′-GMP and L-HMet in a 1 : 1 : 1 mol ratio at pH* 7, after 4, 6 and 24 h. The S-methyl peak of L-HMet (SMe) initially decreases in intensity with time, but by 24 h has increased in intensity again indicating that bound L-HMet is displaced during the reaction

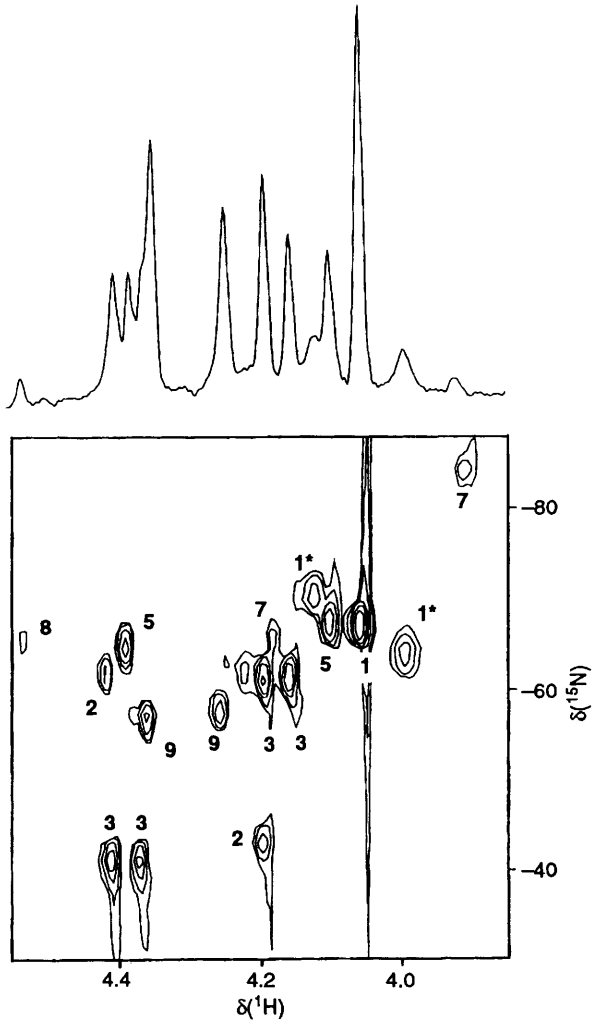


Fig. 3 The 500 MHz 2D [^{1}H, ^{15}N] HMQC NMR spectrum of a solution of cisplatin, 5′-GMP and L-HMet in 1 : 1 : 1 mol ratio after 4 h at 37 °C. Assignments of peaks are given in Table 1. ^{15}N-Decoupling was employed in the ^{1}H dimension and peaks labelled **1*** are ^{195}Pt satellites for cisplatin [$^1J(^{195}\text{Pt}-^{15}\text{N}) = 271$, $^2J(^{195}\text{Pt}-\text{NH}) = 65$ Hz]. The doubling of peaks for **3** and **9** is as expected for slow inversion of co-ordinated S of chelated L-Met

constants were determined: $k = 9.0 \times 10^{-4}$ s^{-1}, with L-HMet present, and 5.4×10^{-4} s^{-1} without. Two additional H^8 peaks were observed in spectra of reactions containing L-HMet (δ 8.80 and 8.79) suggesting that mixed-ligand GMP–L-HMet species are formed.

Up to four major peaks (5′-GMP H^8 peaks) were observed in the aromatic region at any one time. Two of them have shifts similar to *cis*-[PtCl(5′-GMP-N^7)$(NH_3)_2$]$^+$ **5** (δ 8.65) and *cis*-[Pt(5′-GMP-N^7)$_2(NH_3)_2$]$^{2+}$ **6** (δ 8.70). The peak at δ 8.65 eventually disappeared (not present after 8 h) while the peak at δ 8.70 was still present after 24 h. The other two peaks (δ 8.82, 8.83) appeared at the same time (30 min) and continued to grow together and coalesced in spectra recorded at higher temperature (45 °C). This suggests that they may belong to the same species. The species which gives rise to these peaks is the major 5′-GMP-containing product of this reaction.

For the first 8 h of the reaction, the S-methyl peak of unbound L-HMet continued to decrease in intensity, but between 8 and 24 h this peak increased in intensity indicating that methionine which was bound to platinum was being released (Fig. 2). Reversible binding of L-HMet was not observed in the reaction of cisplatin and L-HMet alone, and when less than 1 equivalent of 5′-GMP was used in the reaction (*e.g.* 0.5 mol equivalent), then the amount of reversible binding decreased. Hence it appeared that 5′-GMP could displace co-ordinated L-HMet.

To assist in the identification of the products, ^{15}N spectra were recorded. For direct detection of ^{15}N, higher concentrations of reactants were required (30 mM). To achieve this, gentle initial heating (*ca.* 40 °C, 20 min) was used to effect dissolution of all the cisplatin, resulting in faster reactions. The ^{15}N chemical shift of an ammine co-ordinated to platinum is dependent on the nature of the ligand co-ordinated *trans* to the ammine.[6] An ammine *trans* to sulfur (L-HMet) would be expected to give a peak in the region δ −40 to −50, *trans* to nitrogen (5′-GMP, L-Met) or chloride, δ −55 to −70, or *trans* to oxygen donors (water, hydroxide, carboxylate), δ −80 to −90.

Nitrogen-15 peaks were observed at δ −41.8, −43.8 and −44.3 indicative of H_3NPt groups *trans* to sulfur. There was extensive overlap of signals in the region for ammine *trans* to nitrogen/chloride, making identification of species difficult, although a peak at δ −65.4 confirmed the formation of *cis*-[Pt(5′-GMP-N^7)$_2(NH_3)_2$]$^{2+}$ **6** in the reaction.[14]

Two-dimensional [^{1}H, ^{15}N] *NMR*.—Due to the extensive overlap in the ^{15}N spectra, the reaction was then followed using two-dimensional [^{1}H, ^{15}N] HMQC NMR spectroscopy, which provided the extra resolution of peaks in the ^{1}H dimension. A typical [^{1}H, ^{15}N] spectrum of the reaction mixture recorded after 4 h is shown in Fig. 3. As can be seen, this is still a complicated spectrum to interpret. However a few signals can be assigned on the basis of previous work, such as that for *cis*-[$PtCl_2(NH_3)_2$] **1** (see Table 1), the starting material, which is present for up to 8 h. Another species which is present is

Table 1 Proton NMR chemical shifts for H^8 of guanosine 5′-monophosphate and SMe of L-methionine together with ^1H and ^{15}N NMR chemical shifts of $^{15}NH_3$ ligands

Compound	pH	$\delta(^{15}N)$	*trans*-Ligand	$\delta(^1H)$ NH_3	H^8	SMe
$[PtCl_2(NH_3)_2]$ **1**		−67.6	Cl	4.06	—	—
$[Pt(5'\text{-GMP-}N^7)(L\text{-HMet-}S)(NH_3)_2]^{2+}$ **8**	4.00	−44.3	S	4.48	8.56	2.28
		−67.9	N	4.55		
	7.40	—*	S	—*	8.65	2.28
		−67.9	N	4.59		
$[Pt(L\text{-Met-}S,N)(NH_3)_2]^+$ **3**	7.00	−41.8	S	4.43, 4.38	—	2.53
		−61.8	N	4.17, 4.21	—	2.55
$[Pt(L\text{-Met-}S,N)(5'\text{-GMP-}N^7)(NH_3)]^+$ **9**	7.00	−57.3	N	4.34	8.82	2.65
		−57.9	N	4.24	8.83	2.67
	3.85	−60.6	N	4.24	8.79	2.65
		−59.5	N	4.17	8.80	2.67
$[PtCl(L\text{-HMet-}S)(NH_3)_2]^+$ **2**	7.00	−44.3	S	4.20	—	2.48
		−65.0	Cl	4.44		
$[PtCl(5'\text{-GMP-}N^7)(NH_3)_2]^+$ **5**	7.00	−66.4	N/Cl	4.38	8.65	—
		−69.1	N/Cl	4.09		
$[Pt(5'\text{-GMP-}N^7)_2(NH_3)_2]^{2+}$ **6**	7.00	−65.4	N	4.67	8.70	—

* Peak shifts under water peak when pH is raised.

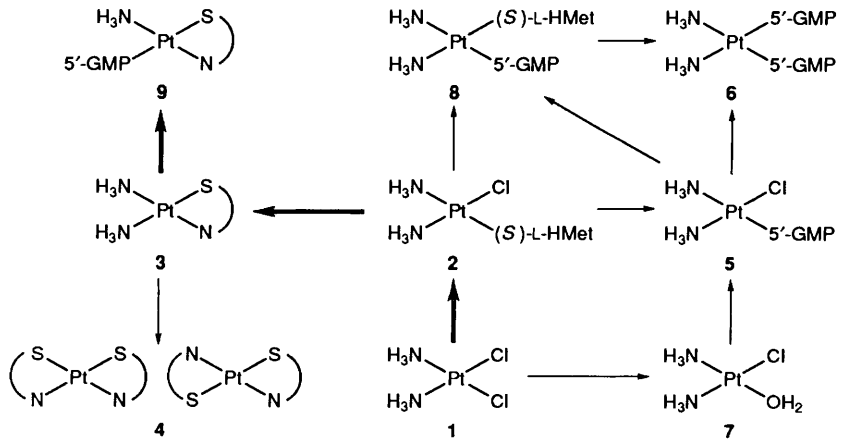
cis-$[PtCl(NH_3)_2(H_2O)]^+$ {or $[PtCl(NH_3)_2(OH)]$} **7**, a hydrolysis product of cisplatin. This product is present for the first 4 h of the reaction and never accounts for more than about 5% of the total products (by peak integration). The spectra also confirm the formation of *cis*-$[PtCl(5'\text{-GMP-}N^7)(NH_3)_2]^+$ **5** during the reaction, (cross-peaks at δ −69.1/4.09 and −66.4/4.38).[12]

Further assignments of two-dimensional [^1H, ^{15}N] cross-peaks were achieved by comparisons with model complexes. Firstly $[Pt(L\text{-Met-}S,N)(NH_3)_2]^+$ **3** was investigated. While the ^{15}N spectrum of this complex has been reported by Appleton *et al.*,[6] the NH proton shifts have not been determined. These workers showed that the ^{15}N spectrum of **3** consisted of four signals, two in the ammine-*trans*-to-sulfur region and two in the ammine-*trans*-to-nitrogen region, assignable to the two diastereoisomers which arise from slow inversion of chiral co-ordinated sulfur. Similar results were obtained here (see Table 1).

The next complex to be investigated was *cis*-$[PtCl(L\text{-HMet-}S)(NH_3)_2]^+$ **2**, a complex which contains monodentate methionine and which is a possible precursor to the chelated complex $[Pt(L\text{-Met-}S,N)(NH_3)_2]^+$ **3**. To synthesise this complex, L-HMet was added to a solution containing *cis*-$[PtCl(NH_3)_2(H_2O)]^+$ **7**. The ^1H NMR spectrum was recorded quickly and it was the major product, together with a small amount of *cis*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ and there was a strong singlet at δ 2.48 assignable to a S-methyl group of L-methionine co-ordinated to platinum as in *cis*-$[PtCl(L\text{-HMet-}S)(NH_3)_2]^+$ **2**. Smaller peaks due to $[Pt(L\text{-Met-}S,N)(NH_3)_2]^+$ **3** were also observed. The two-dimensional [^1H,^{15}N] HMQC spectrum was dominated by two strong peaks of equal intensity at δ −65.0/4.44 and −44.3/4.20 (Table 1). These appeared at the same time and later decreased in intensity together. It was therefore assumed that they belong to two ammine groups co-ordinated to the same platinum atom, one assignable to a co-ordinated ammine *trans* to sulfur (L-HMet), the other to an ammine *trans* to nitrogen/chloride. The only nitrogen donor present in the solution was the amine of L-HMet, and since the complex is not $[Pt(L\text{-Met-}S,N)(NH_3)_2]^+$ **3** (see above) this signal must be due to an ammine-*trans*-to-a-chloride ion and can be assigned to *cis*-$[PtCl(L\text{-HMet-}S)(NH_3)_2]^+$ **2**. When the solution containing this complex was allowed to stand, peaks due to $[Pt(L\text{-Met-}S,N)(NH_3)_2]^+$ **3** increased in intensity, although after 24 h significant quantities of **2** were still present in solution. From these data it is evident that **2** is one of the first products formed on reaction of cisplatin with 5′-GMP and L-HMet, and is present in the reaction for up to 24 h.

Next we characterised the mixed-ligand complex *cis*-$[Pt(5'\text{-GMP-}N^7)(L\text{-HMet-}S)(NH_3)_2]^{2+}$ **8** by ^1H and [^1H,^{15}N] NMR. A solution containing predominately *cis*-$[Pt(5'\text{-GMP-}N^7)(NH_3)_2(H_2O)]^{2+}$ was formed by adding 1 mol equivalent of a pH 4 solution of 5′-GMP to a pH 4 solution of *cis*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$. This pH was chosen to avoid formation of hydroxo species. To this solution was added 1 mol equivalent of L-HMet. The ^1H NMR spectrum showed a new H^8 peak at δ 8.56 which shifted to δ 8.65 when the pH* of the solution was raised from 4.0 to 7.4. A new singlet assignable to the S-methyl of bound L-HMet was also observed at δ 2.28. This chemical shift is much smaller than is normally observed for S-methyl groups co-ordinated to platinum (δ 2.4–2.7) and which may be due to ring-current effects arising from a purine ring co-ordinated *cis* to 5′-GMP as in **8**. The two-dimensional [^1H, ^{15}N] HMQC spectrum (see Table 1 for shifts) showed two new cross-peaks of equal intensity, one with shifts characteristic of an ammine *trans* to sulfur (L-HMet), the other characteristic of ammine *trans* to nitrogen. Since this nitrogen is not the amine of L-HMet (see above), it must be N of 5′-GMP. When the pH of the solution was raised from 4.0 to 7.4, there was a significant change in the spectrum. The cross-peak due to the ammine *trans* to 5′-GMP shifted very little but the peak due to the ammine *trans* to the sulfur disappeared and was assumed to be beneath the water peak. Similar low field ^1H NMR shifts have been observed previously for ammine groups co-ordinated *cis* to 5′-GMP ligands.[12] The increase in pH is likely to result in deprotonation of the phosphate group to give the dianionic form, which, in turn, may lead to a change in hydrogen bonding between the phosphate group and the *cis*-NH_3 group and to the low-field shift of the NH resonance.

It is difficult to assign peaks for the mixed-ligand complex **8** in normal ^1H NMR spectra of the cisplatin–5′-GMP–L-HMet reaction mixture, because its H^8 signal is expected to coincide with the H^8 signal of *cis*-$[PtCl(5'\text{-GMP-}N^7)(NH_3)_2]^+$ **5** and the S-methyl signal would be buried beneath the various signals of the β-CH_2 protons of co-ordinated and unco-ordinated L-methionine. The two-dimensional [^1H, ^{15}N] spectrum of the reaction mixture contained a small cross-peak at δ −67.9/4.59 which may correspond to the ammine *trans* to 5′-GMP in



Scheme 1 A possible scheme for the reaction of cisplatin with 5'-GMP in the presence of L-HMet. The thickened arrows connect the major observed intermediates and products. The charges on complexes have been omitted

cis-[Pt(5'-GMP-N^7)(L-HMet-*S*)$(NH_3)_2$]$^{2+}$ **8** but, as the pH of the reaction mixture is 7.0, the signal for the ammine *trans* to sulfur is likely to be under the water resonance and so not observable.

Labilization of NH_3.—Previous reports have indicated that a co-ordinated sulfur atom of L-HMet can labilize an ammine group co-ordinated in the *trans* position.[6,7] Therefore [Pt(L-Met-*S*,*N*)$(NH_3)_2$]$^+$ **3** (prepared as described above) was reacted with 5'-GMP in a 1 : 1 mol ratio at pH* 7.00. Two new ^{1}H NMR peaks at δ 8.79 and 8.80 assignable to H^8 of 5'-GMP species and two at δ 2.65 and 2.67 assignable to S-methyls of L-HMet products were observed after 1 h. These H^8 peaks coalesced when the temperature was raised from 37 to 45 °C. The two-dimensional [^{1}H, ^{15}N] spectrum showed two new peaks in the ammine-*trans*-to-nitrogen region. No peaks in the ammine-*trans*-to-sulfur region were observed. The proton spectrum is consistent with the co-ordination of L-HMet through the sulfur atom, and the lack of a cross-peak for an ammine *trans* to sulfur in the two-dimensional [^{1}H, ^{15}N] spectrum may be explained by the replacement of the ammine *trans* to the sulfur by another group. The presence of two sets of signals in both the ^{1}H and in the two-dimensional [^{1}H, ^{15}N] NMR spectra is consistent with formation of a species containing chelated L-Met with slow inversion of the S-methyl group on the NMR time-scale. These data are consistent with [Pt(L-Met-*S*,*N*)(5'-GMP-N^7)(NH_3)]$^+$ **9** being a product from the reaction of [Pt(L-Met-*S*,*N*)$(NH_3)_2$]$^+$ **3** with 5'-GMP. When the pH of the solution was lowered to *ca* 3.85 high field shifts were observed in the two-dimensional [^{1}H, ^{15}N] spectrum, consistent with a change in H-bonding between 5'-GMP and an ammine co-ordinated in a *cis* position (see above).

We conclude that [Pt(L-Met-*S*,*N*)(5'-GMP-N^7)(NH_3)]$^+$ **9** is also formed in the reaction of cisplatin with L-HMet and 5'-GMP (Fig. 3). Both ^{1}H and two-dimensional [^{1}H, ^{15}N] NMR spectra indicate that **9** is the major 5'-GMP product formed in this reaction. The formation of this product may explain the increase in the rate of reaction of 5'-GMP with cisplatin in the presence of L-HMet. The labilization of NH_3 *trans* to S in *cis*-[PtCl(L-HMet-*S*)$(NH_3)_2$]$^+$ **2** and [Pt(L-Met-*S*,*N*)$(NH_3)_2$]$^+$ **3** provide alternative pathways for 5'-GMP binding.

The model studies therefore lead to the identification of most of the products seen in the reaction of cisplatin with 5'-GMP and L-HMet in a 1 : 1 : 1 mol ratio at pH 7, and the reactions shown in Scheme 1 can be proposed. As the formation of the major product of the reaction involves the labilization of an ammine ligand, the reaction of [Pt([^{15}N]-en)Cl_2] which contains the less labile chelating ligand 1,2-diaminoethane, with 5'-GMP and L-HMet (1 : 1 : 1) was studied under the same conditions for comparison.

Reactions of [Pt([^{15}N]-en)Cl_2] *with* L-HMet *and* 5'-GMP.—It was found that in the presence of L-HMet much less 5'-GMP (< 15%) reacted with [Pt([^{15}N]-en)Cl_2] than cisplatin in the corresponding reaction, consistent with the more rapid chelation of methionine and the lack of labilization of chelated en. The complex [Pt(en)(5'-GMP-N^7)$_2$]$^{2+}$ was the major 5'-GMP product, a species which has been characterized previously.[12] Both ^{1}H and two-dimensional [^{1}H, ^{15}N] NMR spectra showed that the major product (> 85%) of this reaction is [Pt(en)(L-Met-*S*,*N*)]$^+$ **10**. A singlet at δ 2.62 in the ^{1}H NMR spectrum can be assigned to the S-methyl group of **10**, and the two-dimensional [^{1}H, ^{15}N] NMR spectrum showed two broad cross-peaks in the amine-*trans*-to-nitrogen/chloride region with the same ^{15}N shift (δ −27.4) but different ^{1}H shifts (δ 5.42 and 5.54). Four peaks were observed in the nitrogen-*trans*-to-sulfur region with the same ^{15}N shift (δ −9.1) and ^{1}H shifts of δ 5.46, 5.51, 5.58 and 5.61. This spectrum is consistent with the formation of an S,N chelate since chelation results in slow inversion of the S-methyl group on the NMR time-scale,[6,7] and this gives rise to two diastereomers and two sets of signals, as is observed for [Pt(L-Met-*S*,*N*)$(NH_3)_2$]$^+$ **3**. Unlike in **3**, the NH protons of [Pt(en)(L-Met-*S*,*N*)]$^+$ are magnetically non-equivalent. This results in four ^{1}H/^{15}N cross-peaks being observed in the amine-*trans*-to-sulfur region. Only two were observed in the nitrogen-*trans*-to-nitrogen region, although they were quite broad, suggesting that there is some exchange broadening (smaller shift difference than for amine *trans* to S). As little [Pt(en)Cl(L-HMet-*S*)] was observed during this reaction, chelate ring closure appears to be rapid.

When [Pt(en)(L-Met-*S*,*N*)]$^+$ was treated directly with 5'-GMP, no changes in ^{1}H or [^{1}H, ^{15}N] NMR spectra were observed over a three-week period. This result, along with others,[15,16] suggests that chelated methionine is much less labile than monodentate methionine. This finding may have important implications for the mechanism of action of cisplatin and other platinum anticancer drugs, introducing the possibility of platinum transfer onto DNA *via* displacement of monodentate S-bound methionine, which could be the amino acid itself or part of a peptide or protein.

Conclusion

Sulfur ligands such as in the thioether L-methionine have a very high affinity for Pt^{II}. Indeed they have been used in rescue agents for the removal of platinum from the body after treatment with platinum anticancer drugs. Therefore it is surprising to find that the reaction of 5'-GMP with cisplatin is faster in the presence of L-methionine than in its absence. The reason for this appears to be that the reaction takes a different course. The major product is [Pt(L-Met-

S,N)(5′-GMP-N^7)(NH_3)]$^+$ **9**, which arises *via* labilization of NH_3 from the intermediate complex [Pt(L-Met-*S,N*)(NH_3)$_2$]$^+$ **3** owing to the high *trans* effect of S. The initial stage of the reaction is dominated by the interaction of cisplatin with L-HMet, but displacement of monodentate L-HMet from platinum is then observed. Two monodentate methionine species were identified during the reaction: *cis*-[PtCl(L-HMet-*S*)(NH_3)$_2$]$^+$ **2** and *cis*-[Pt(5′-GMP-N^7)(L-HMet-*S*)(NH_3)$_2$]$^{2+}$ **8**. In contrast, displacement of S,N-chelated methionine does not appear to occur readily at physiological pH. In biological systems the Met residue could be part of a peptide or protein and therefore be less likely to chelate, and displacement of S by guanine bases may be even more favourable. Studies to investigate this are in progress.[17]

Acknowledgements

We thank the MRC, International Association for Cancer Research, Royal Society, Sybil Evans Trust, Biotechnology and Biological Sciences Research Council, EC (Cooperation in Science and Technology and Human Capital Mobility) and University of London Intercollegiate Research Service for their support for this work, and Dr Tom Frenkiel (MRC Biomedical NMR Centre, Mill Hill) for assistance with inverse probe experiments.

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Received 23*rd June* 1995; *Paper* 5/04057D