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Supplementary Material Available: Listings of final positional and anisotropic thermal parameters for $W(\eta^5\text{-}C_5Me_5)Me_4(\eta^2\text{-}NHNH_2)$ (Table S1), final positional and B(eq) parameters for $[W(\eta^5\text{-}C_5Me_5)Me_3(\eta^2\text{-}NHNH_2)]^+[SO_3CF_3]^-$ (Table S2), and anisotropic thermal parameters for $[W(\eta^5\text{-}C_5Me_5)Me_3(\eta^2\text{-}NHNH_2)]^+[SO_3CF_3]^-$ (Table S3) (5 pages); listings of final observed and calculated structure factors for $W(\eta^5\text{-}C_5Me_5)Me_4(\eta^2\text{-}NHNH_2)$ (Table S4) and $[W(\eta^5\text{-}C_5Me_5)Me_3(\eta^2\text{-}NHNH_2)]^+[SO_3CF_3]^-$ (Table S5) (52 pages). Ordering information is given on any current masthead page.

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^{14}N NMR Studies of Amine Release from Platinum Anticancer Drugs: Models and Human Blood Plasma[1]

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The feasibility of using $^{14}N\{^1H\}$ NMR spectroscopy to follow reactions of Pt(II) antitumor drugs under biologically relevant conditions has been investigated. Amine release from *cis*-$PtCl_2(NH_3)_2$ upon reaction with both L-methionine and *N*-acetyl-L-methionine and from $PtCl_2$(1,2-diaminoethane) on reaction with L-methionine in aqueous solution can be readily detected. Upon incubation (37 °C for 24 h) of *cis*-$PtCl_2(NH_3)_2$ with human blood plasma supplemented with L-methionine, at least one NH_3 ligand appears to be lost. Ammonia release is also detected upon addition of excess sodium diethyldithiocarbamate (an agent used clinically to reverse cisplatin toxicity) to plasma incubated with *cis*-$PtCl_2(NH_3)_2$ (37 °C for 2 h). Other ^{14}N peaks assigned in plasma spectra include those for amides, phosphatidylcholines, and N_2. We conclude that ^{14}N NMR spectroscopy provides a useful probe for studying these drugs at millimolar concentrations under conditions that approach physiological relevance.

Introduction

There is current interest in understanding the chemical basis for the mechanism of action of the antitumor drug cisplatin,[3] *cis*-$PtCl_2(NH_3)_2$, and its toxic side effects. Although current evidence suggests that the important cytotoxic lesion is an intrastrand cross-link arising from *cis*-$Pt(NH_3)_2^{2+}$ binding to DNA,[4] ammonia can be liberated from cisplatin under mild conditions.[5] Naturally occurring sulfur-containing ligands such as methionine and cysteine are particular candidates for study because, on substitution for Cl^- in cisplatin, their high kinetic trans effects[6] may induce rapid NH_3 release.[7] Methionine-containing Pt(II) metabolites have apparently been isolated from the urine of patients receiving cisplatin,[8] and numerous studies have been conducted on blood plasma.[9] However, previous attempts to identify metabolites formed from cisplatin in biological materials have involved chemical separation and isolation procedures[8,9] during which further transformation could occur. We are therefore exploring methods for the speciation of Pt (and other biologically relevant metals) in *intact* biological materials.

The detection limit for many small molecules in ^{1}H NMR spectra is now as low as 100 μM, and the background of interfering resonances can often be filtered out via suitable pulse sequences. We have recently shown[10] by ^{1}H NMR spectroscopy that cisplatin, $PtCl_2$(en), and K_2PtCl_4 react with L-Met in cell culture media at 37 °C to produce new species, one of which appears to be Pt(L-Met-*N,S*)$_2$. In this paper we explore the use of ^{14}N NMR spectroscopy for the direct detection of amine release in human

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(3) The following abbrevations are used throughout the text: cisplatin, *cis*-$Pt(NH_3)_2Cl_2$; NaDEDTC, sodium diethyldithiocarbamate; en, 1,2-diaminoethane; L-Met, L-methionine; *N*-Ac-L-Met, *N*-acetyl-L-methionine; NMR, nuclear magnetic resonance.

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blood and in model systems. In particular we have examined reactions with L-Met and with the sodium salt of diethyldithiocarbamate (DEDTC), a ligand that has been advocated for clinical use to reverse cisplatin toxicity and to aid in overdose recovery.[11] We have also extended the studies to the release of the chelated diamine from $PtCl_2(en)$, which is also an active antitumor agent.[12]

The use of ^{14}N NMR spectroscopy has been relatively little explored in biological chemical studies.[13] ^{14}N (99.63% abundance) is quadrupolar with a nuclear spin $I = 1$. This often leads to broad NMR signals,[14] perhaps accounting for its infrequent use since natural-abundance ^{15}N ($I = ^1/_2$) NMR is now accessible and isotopic ^{15}N enrichment often possible. However, in a preliminary experiment to detect NH_3 release from *cis*-$PtCl_2(^{15}NH_3)_2$ in blood plasma under conditions similar to those described below, we were unable to detect resonances for $^{15}NH_4^+$. This may have been due to unfavorable nuclear Overhauser effects (the gyromagnetic ratio of ^{15}N is negative; hence, the NOE enhancement is negative) since correlation times are unknown. To our knowledge, there have been no reports of the use of ^{14}N NMR spectroscopy to study blood, blood plasma, or ultrafiltrates. Although a number of biologically relevant chemical species have been studied by ^{14}N NMR spectroscopy,[15] typically only urea and highly symmetrical species such as NH_4^+ and $RN(CH_3)_3^+$ groups are observed in ^{14}N spectra of biological samples such as mammalian tissue, tissue homogenates,[16] and pea and barley roots.[17] Thus, ^{14}N NMR spectroscopy seemed to offer the possibility of detecting ammonia release from cisplatin both in chemically defined mixtures and in more complicated media such as human blood plasma. To approach biological relevance, it was considered important to attempt the reactions at submillimolar concentrations.

Experimental Section

NMR Measurements. ^{14}N NMR spectra were collected at ambient temperature (ca. 294 K) on a Bruker AM400 spectrometer (MRC Biomedical NMR Center, Mill Hill, U.K.) operating at 28.9 MHz and using 10-mm tubes. Spectra were obtained by using a 20-μs pulse (60° flip angle), 32K data points, a 20-kHz spectral width, a 1.5-s pulse repetition rate, quadrature phase detection, and composite pulse decoupling. A 30-Hz line-broadening function was applied to all free induction decays before Fourier transformation. Chemical shifts are referenced to neat external CH_3NO_2. In some cases secondary chemical shift references were used, either $NaNO_3$ at −5.4 ppm or natural $RN(CH_3)_3^+$ choline lipoprotein head groups of phospholipids at −334 ppm.

^1H NMR spectra were collected at ambient temperature on a Bruker AM500 spectrometer (MRC Biomedical NMR Center) operating at 500 MHz and using 5-mm tubes. Spectra were obtained by using 16K data points, a 3-s repetition rate, quadrature detection, and collection of 32–48 free induction decays. Hahn spin-echo spectra were collected by using a t value of 60 ms in the 90-t-180-t-collect sequences.[18] The intense signal from H_2O was suppressed by the application of secondary irradiation. Typically a 1.0-Hz exponential line-broadening function was applied to the free induction decay prior to Fourier transformation. Chemical shifts are referenced to the low-frequency doublet of valine (1.050 ppm) or the doublet of lactate (1.330 ppm).

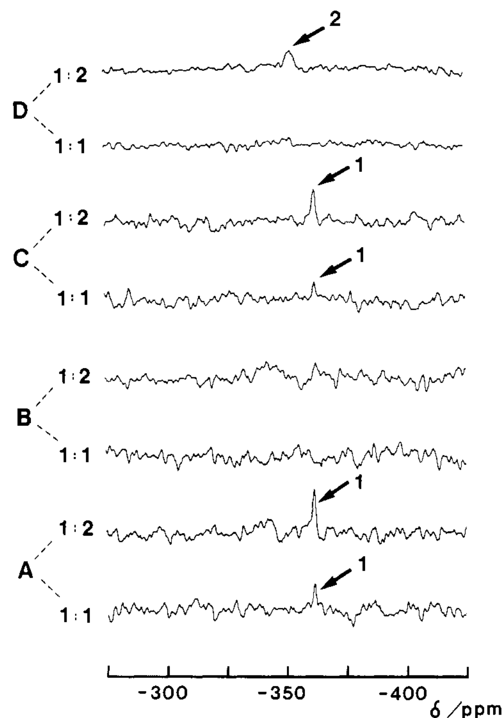

Figure 1. 28.9-MHz $^{14}N\{^1H\}$ NMR spectra of (A) L-Met and *cis*-$PtCl_2(NH_3)_2$ (2 mM in 0.15 M NaCl) at 1:1 and 2:1 mole ratios, both 800 transients, (B) L-Met and *trans*-$PtCl_2(NH_3)_2$ at 1:1 and 2:1 mole ratios, both 1200 transients, (C) *N*-Ac-L-Met and *cis*-$PtCl_2(NH_3)_2$ (2 mM in 0.15 M NaCl) at 1:1 and 2:1 mole ratios, both 1200 transients, and (D) L-Met and $PtCl_2(en)$ (2 mM in 0.15 M NaCl) at 1:1 and 2:1 mole ratios, both 2400 transients. All solutions were incubated at 37 °C for 24 h. The signal-to-noise ratios are relatively poor, but note the low concentrations of reactants. Peak assignments: 1, NH_4^+; 2, $H_3NCH_2CH_2NH_3^{2+}$.

Blood Plasma. Blood was donated by healthy female and male volunteers, collected in standard vials containing lithium heparin (anticoagulant), and centrifuged to remove red cells. Portions of the resulting plasma were filtered through an Amicon 10 kDa cutoff centrifugal filter.

Reaction of Cisplatin in Blood Plasma: ^{14}N NMR Studies. A solution of 0.1 mM cisplatin (Aldrich) (8 mL in 0.15 M NaCl to inhibit hydrolysis) was lyophilized. Blood plasma (1.8 mL) was added to the resulting solid, which all dissolved. To this solution was added 0.2 mL of 2 mM L-Met (Sigma) in ^2H_2O, and the sample was incubated at 37 °C for 24 h prior to the collection of its ^{14}N NMR spectrum. The total accumulation time (at ambient temperature) was 11 h; thus, the average incubation time corresponding to the spectrum was 24 h at 37 °C plus 5.5 h at 21 °C. Similarly, as a control, 8 mL of 0.15 M NaCl was lyophilized and 1.8 mL of blood plasma was added to the resulting solid, followed by the addition of 0.2 mL of 2 mM L-Met in ^2H_2O and incubation at 37 °C for 24 h. The accumulation time was the same. The final concentration of cisplatin was 0.4 mM, and the concentration of added L-Met was 0.2 mM.

Reactions of $PtCl_2(NH_3)_2$ in Blood Plasma: ^1H NMR Studies. Fresh blood plasma or ultrafiltrate (0.45 mL) was placed in a 5-mm NMR tube and 2 mM L-Met (0.05 mL of a 2 mM solution in ^2H_2O) added to provide a lock signal. Suitable concentrations of Pt(II) complexes were achieved by lyophilizing an appropriate volume of 0.1 mM stock solution (containing 150 mM NaCl to inhibit hydrolysis) and redissolving the solids in 0.45 mL of plasma or ultrafiltrate. All samples were incubated at 37 °C, and spectra were recorded at various times up to 28 h. The final concentrations of *cis*- and *trans*-$PtCl_2(NH_3)_2$ were 0.2 and 0.4 mM, respectively. The final added L-Met concentration was 0.2 mM.

Reaction of Cisplatin with DEDTC: ^{14}N NMR Studies. Cisplatin (0.62 mg, 2 μmol) was dissolved in 2 mL of blood plasma and incubated

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Table I. $^{14}N\{^1H\}$ NMR Chemical Shifts and Line Widths

compd	δ^a	$\Delta\nu_{1/2}$/Hz	compd	δ^a	$\Delta\nu_{1/2}$/Hz
$NaNO_3$[b]	−5.4	12	L-Met[d]	−343	130
N_2	−72	10	en[e]	−352	60
NaDEDTC[c]	−201	560	cisplatin[f]	−430	70

[a] Chemical shifts referenced to external neat CH_3NO_2. [b] 100 mM in ^2H_2O. [c] $Na(S_2CNEt_2)\cdot 2H_2O$ in ^2H_2O. [d] L-Met in ^2H_2O. [e] 1,2-Diaminoethane dihydrochloride in ^2H_2O. [f] Saturated solution of cisplatin in ^2H_2O.

at 37 °C for 2 h. DEDTC, sodium salt (4.59 mg, 20 μmol; Sigma), and 0.2 mL of ^2H_2O (as lock) were added, and the spectrum was recorded immediately. Total accumulation time was 11 h. Similarly, as a control, NaDEDTC (4.71 mg) and ^2H_2O (0.2 mL) were added to 2 mL of plasma, and the spectrum was recorded with the same total spectral accumulation time.

NaDEDTC (9.07 mg, 0.04 mmol) and cisplatin (6.13 mg, 0.02 mmol) were dissolved in 5 mL of ^2H_2O. The solution was heated at ca. 70 °C for ca. 3 min (with the formation of a yellow-green precipitate) and filtered through washed cotton, and its spectrum was recorded.

Reaction of $PtCl_2(NH_3)_2$ and $PtCl_2(en)$ with L-Met: ^{14}N NMR Studies. Various solutions containing 1:1 and 2:1 L-Met to Pt mole ratios were prepared in 150 mM saline (to inhibit hydrolysis) from cisplatin, *trans*-$PtCl_2(NH_3)_2$, and $PtCl_2(en)$. A typical preparation was made as follows: Cisplatin (3.02 mg, 10.1 μmol), L-Met (3.08 mg, 20.6 μmol), and NaCl (43.76 mg, 0.749 mmol) were dissolved in 5 mL of ^2H_2O, and the solution was incubated at 37 °C for 24 h. The final pH was 6.5. $NaNO_3$ solution (final concentration 10 mM) was added as a chemical shift reference immediately prior to spectral accumulation. Under these conditions, all of the trans complex did not dissolve.

Results

Model Reactions. Figure 1 shows the ^{14}N NMR spectra resulting from reactions (24 h, 37 °C, nearly neutral pH) of L-Met and *N*-Ac-L-Met with Pt(II) complexes in 0.15 M NaCl. Ammonium ions are produced on reaction of L-Met with cisplatin in 1:1 and 2:1 ratios (Figure 1A) but not from the reaction of L-Met with the trans complex (Figure 1B). Similarly, reaction of *N*-Ac-L-Met with cisplatin also leads to the production of ammonium ions (Figure 1C), a result noted previously by ^{15}N NMR spectroscopy.[5e] The reaction of L-Met with $PtCl_2(en)$ resulted in amine release (peak for enH_2^{2+} at −352 ppm) at a 2:1 ratio but not at a 1:1 ratio (Figure 1D). The reaction of DEDTC with cisplatin (2:1 ratio) resulted in the formation of a yellow-green precipitate, but NH_4^+ was readily detected in the supernatant by $^{14}N\{^1H\}$ NMR. No peaks attributable to unreacted Pt complexes were observed, nor were peaks observed for free L-Met ($-NH_3^+$ at −343 ppm), free DEDTC ($-N(CH_2CH_3)_2$ at −201 ppm), coordinated L-Met, or coordinated DEDTC.

Plasma. The relevant regions of the $^{14}N\{^1H\}$ NMR spectra of the control plasma and the plasma with added cisplatin are shown in Figure 2. The ^{14}N chemical shifts of a number of chemical species are collected in Table I. The appropriate choice of chemical shift standard is difficult for studies of NH_4^+ in blood plasma at submillimolar levels by ^{14}N NMR spectroscopy. The use of the commonly accepted standard (neat external CH_3NO_2) resulted in a dynamic range problem even when a 1-mm capillary was used. In an attempt to provide an internal chemical shift reference as well as a concentration standard, a solution of $NaNO_3$ (final concentration 10 mM) was added to plasma. However, the resulting line width was 217 Hz, nearly broadened beyond detection. In comparison, the line width of 100 mM $NaNO_3$ in ^2H_2O was only 12 Hz.

The peak at −334 ppm (which appeared in all our plasma spectra and was a useful chemical shift reference) has a shift comparable to that of choline.[15,16] Yet this peak is absent from the ^{14}N NMR spectrum of plasma ultrafiltrate (spectrum not shown), suggesting that it is due to a choline-like group of a macromolecule (molecular mass > 10 kDa), such as the phosphatidylcholines of lipoproteins. The peak at ca. −310 ppm is assigned to amides such as urea. It is quite broad in plasma (line width ca. 400 Hz) yet considerably narrowed in plasma ultrafiltrate (ca. 150 Hz wide), perhaps suggesting[16a] that urea is associated with proteins in plasma or that it is a composite peak

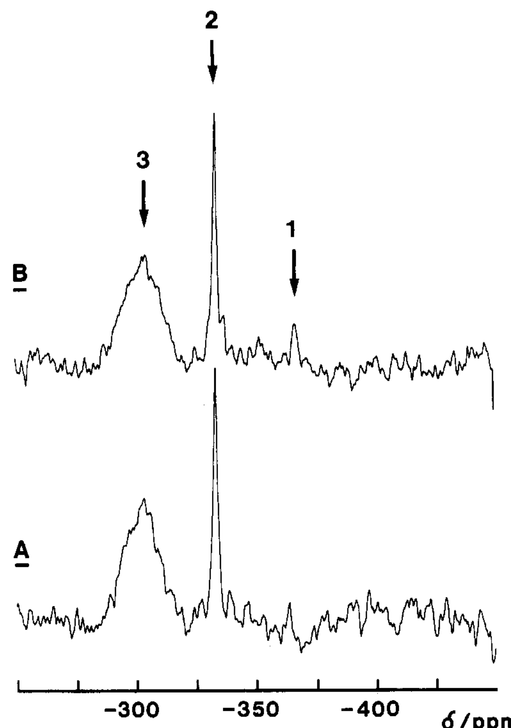

Figure 2. 28.9-MHz $^{14}N\{^1H\}$ NMR spectra of (A) human blood plasma containing 0.2 mM added L-Met (36089 transients) and (B) sample described in part A with 0.4 mM *cis*-$PtCl_2(NH_3)_2$ added (46600 transients). Peak 1 is assigned to NH_4^+ displaced from cisplatin, and peaks 2 and 3 are assigned to $-NMe_3^+$ choline head groups of phospholipids (mainly in low-density and high-density lipoproteins, LDL and HDL) and amides, respectively.

with contributions from high molecular weight amides. An additional peak was occasionally observed at −72 ppm (spectral region not shown) (Table I). This had been previously noted in ^{14}N NMR spectra but not assigned.[17]

Since a concentric capillary insert filled with air gave rise to the signal at −72 ppm, we have assigned it to N_2. No peak for the amino group of L-Met added to blood plasma is resolved (−343 ppm; Table I). A distinct new peak appeared near −362 ppm after reaction of cisplatin with plasma (Figure 2B). Few chemical species of nitrogen, apart from NH_4^+, are symmetrical enough to give rise to relatively sharp ^{14}N signals and have resonances in this region of the spectrum. Consequently, we conclude that the reaction of cisplatin had led to the production of NH_4^+. The line width of this signal is 83 Hz. No peak for cisplatin (−430 ppm; Table I) is apparent.

The peak for NH_4^+ is more pronounced in Figure 3, in which ^{14}N NMR spectra of plasma with added DEDTC in both the presence and the absence of cisplatin are compared. The plasma had been incubated with cisplatin (1 mM) for 24 h at 37 °C prior to addition of DEDTC (9.1 mM) to mimic a clinically relevant rescue situation. The plasma was clear after addition of DEDTC, but a precipitate had formed (similar in appearance to that from direct reaction of DEDTC with cisplatin; see Experimental Section) by the end of the NMR accumulation (11 h at 21 °C). The line width of the NH_4^+ signal is 62 Hz. No peaks for DEDTC (−201 ppm; Table I) or cisplatin (−430 ppm) were observed.

In an attempt to quantify ammonia release, standard additions of NH_4Cl (1 and 10 mM) were made to plasma and integration was performed with respect to the choline-like signal at −334 ppm. The resulting NH_4^+ line widths were 29 and 17 Hz, respectively. Comparison of the spectra of these standard additions with the spectra shown in Figures 1 and 2 suggested that the concentrations of NH_4^+ produced upon addition of 0.4 mM cisplatin to L-Met-enriched plasma and upon addition of a 10-fold excess of DEDTC to plasma containing 1 mM cisplatin were 0.25 and 0.79 mM, respectively. This implies that at least one of the ammonia ligands is lost from cisplatin (1.3 and 1.6, respectively) in these reactions. These calculations do not include any allowance for

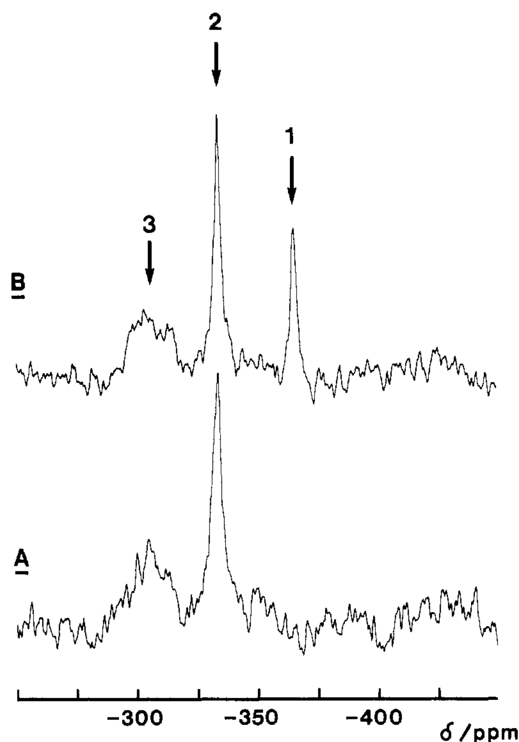

Figure 3. 28.9-MHz $^{14}N\{^1H\}$ NMR spectra of (A) human blood plasma containing 10 mM added NaDEDTC (41 800 transients) and (B) sample described in A with 1 mM added *cis*-$PtCl_2(NH_3)_2$ (41 800 transients). Peak 1 is assigned to NH_4^+ resulting from displacement of NH_3 from cisplatin on reaction with DEDTC, and peaks 2 and 3 are assigned to choline head groups of phospholipids and amides, respectively.

possible NOE effects (usually small with quadrupolar nuclei) or variations in the composition of the choline-like peak.

^{1}H NMR Studies. The 500-MHz ^{1}H NMR Hahn spin-echo spectra of blood plasma and plasma ultrafiltrate at various times after addition of *cis*- and *trans*-$PtCl_2(NH_3)_2$ are shown in Figure 4. The spin-echo sequence acts as a filter for the broad peaks in the spectrum (mainly from proteins and lipoproteins). During the $2t$ delay period in the sequence (120ms), the magnetization associated with these protons (T_2 values < ca. 40 ms) decays away, and so simpler and more easily interpretable spectra are obtained. Singlets (e.g. $S-CH_3$ of Met) remain upright, but coupled multiplets undergo phase modulation. Addition of cisplatin results in a decrease in intensity of the L-Met $S-CH_3$ and $S-CH_2$ peaks, indicative of an interaction of L-Met with cisplatin. In contrast, *trans*-$PtCl_2(NH_3)_2$ does not affect the L-Met $S-CH_3$ singlet of plasma even after 28 h at 37 °C. In plasma ultrafiltrates (i.e. protein-free), however, addition of *trans*-$PtCl_2(NH_3)_2$ results in a more rapid decrease in intensity of the L-Met $S-CH_3$ and $S-CH_2$ peaks compared to the reaction involving cisplatin (Figure 4C–E). The trans complex appeared to reduce the intensity of peaks for histidine (not shown) in the ultrafiltrate spectrum, whereas the cis complex did not. Formation of S-bound Pt–Met complexes would be expected to shift the $S-CH_3$ and $S-CH_2$ resonances to higher frequency by 0.4–0.5 ppm. No new resonances were clearly discernible in these spectra (unlike those of cell culture media[10]), perhaps due both to the low concentrations of the products and to their slow tumbling (short T_2's).

Discussion

Ammonia is known to be released from cisplatin under certain mild conditions.[5] Our earlier results[5d-f] with ^{15}N-labeled cisplatin encouraged us to explore the possibility of $^{15}NH_3$ release in human blood plasma. However, we were not successful (see Introduction). Consequently, we decided to investigate the possible use of ^{14}N NMR spectroscopy. First, it was necessary to establish that NH_3 release is observable in chemically defined model systems.

In our model systems, amine release was detectable by ^{14}N NMR spectroscopy upon reaction of $PtCl_2(en)$ with L-Met and

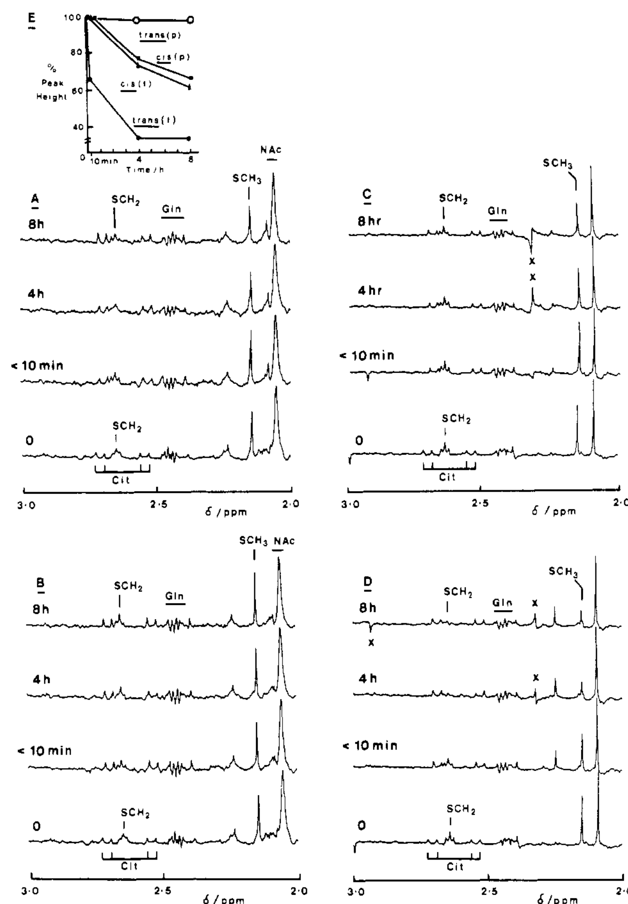

Figure 4. 500-MHz Hahn spin-echo ^{1}H NMR spectra of (A) human blood plasma (supplemented with 0.2 mM L-Met) at various times after addition of *cis*-$PtCl_2(NH_3)_2$ (0.2 mM), (B) sample described in part A but with *trans*-$PtCl_2(NH_3)_2$ (0.4 mM) added, (C) human blood plasma ultrafiltrate (molecular mass < 10 kD; supplemented with 0.2 mM L-Met) at various times after addition of *cis*-$PtCl_2(NH_3)_2$ (0.2 mM), and (D) sample described in part C but with *trans*-$PtCl_2(NH_3)_2$ (0.4 mM) added. Peak assignments: SCH_3 and SCH_2, L-Met $-CH_2-S-CH_3$; Cit, citrate CH_2; Gln, glutamine CH_2; NAc, *N*-acetyl groups of glycoproteins; ×, noise spike. Part E is a plot of the intensity of the SCH_3 resonance versus time; f and p refer to filtrate and plasma, respectively.

upon reaction of cisplatin with L-Met, *N*-Ac-L-Met, and DEDTC. All these reaction mixtures contained 2 mM added Pt(II) and were incubated at 37 °C for 24 h. No NH_3 release was detectable from *trans*-$PtCl_2(NH_3)_2$ upon reaction with L-Met under these conditions.

Cisplatin itself gave rise to a broadened ^{14}N triplet at −430 ppm. The ^{195}Pt–^{14}N coupling of 220 Hz, when scaled by $\delta(^{15}N)/\delta(^{14}N)$ (−1.4028), is equivalent to a $^1J(^{195}Pt-^{15}N)$ of 309 Hz, comparable to the reported values of 312 Hz (in DMSO and H_2O at 30 °C)[5d,e] and 325 Hz (in H_2O at 30 °C)[19] observed for the ^{15}N-labeled drug.

No Pt(II)-containing species were detectable by ^{14}N NMR spectroscopy in any of the model reactions shown in Figure 1. This may have been due to the low concentrations of Pt complexes used and/or broadening of the resonances via quadrupolar relaxation. Thus, L-Met bound to Pt(II) did not give rise to an observable ^{14}N NMR signal.

Therefore, it is possible, as suggested earlier,[5e] that some of the differing biological activities of cis and trans platinum(II) diamine complexes may be related to sulfur-induced amine release. Under the conditions employed here, there was a clear difference in the reactions of *cis*- and *trans*-$PtCl_2(NH_3)_2$ with L-Met in model systems, in blood plasma, and in plasma ultrafiltrates, as shown by ^{14}N and ^{1}H NMR spectroscopy. The results suggest that sulfur of L-Met (and *N*-Ac-L-Met) displaces Cl^- from cisplatin and labilizes the trans ligand. Similarly, for $PtCl_2(en)$ the trans $-NH_2$

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group is labilized, allowing a second L-Met to coordinate and resulting in complete displacement of en, a result observed previously[5e] by ^{15}N NMR spectroscopy for the reaction of $PtCl_2(en)$ with *N*-Ac-L-Met. The lack of reactivity of *trans*-$PtCl_2(NH_3)_2$ with L-Met in intact plasma is surprising. This may arise from its reactivity toward proteins, perhaps involving histidine residues.

There do not appear to be any previous reports of reactions of cisplatin with DEDTC, although Pt(II) is known to react with *N,N'*-dialkyldithiocarbamates. $Pt(DEDTC)_2$ has been studied by a variety of techniques including X-ray crystallography,[20] mass spectrometry,[21] infrared spectroscopy,[22] and ^1H, ^{13}C, and ^{195}Pt NMR spectroscopy.[23] We assume that the precipitate formed from the reaction of DEDTC with cisplatin (2:1) is $Pt(DEDTC)_2$ and that both NH_3 ligands are released under these conditions. However, the precipitate was not further characterized. A similar precipitate appeared to be formed upon addition of excess DEDTC to blood plasma containing cisplatin. The relative affinities of ammonia and dimethyldithiocarbamate (DMDTC) for Pt(II) are evident from the work of Derbisher and co-workers,[24] who noted that the addition of ammonia to platinum(II) tetramethylthiuram disulfide resulted in $Pt(DMDTC)_2$, which did not contain any ammine ligands. Jain and Srivastava[25] have reported the preparation of $[Pt(en)(DEDTC)]^+$ as the nitrate.

Plasma. It is interesting to compare the differences between the $^{14}N\{^1H\}$ NMR spectra shown in Figure 2A and 3A. The plasma for the former was donated by a male volunteer ca. 3 h after a meal, while that for the latter was donated by a female volunteer after an overnight fast. It is evident that the $-N(CH_3)_3^+$ peak is narrower in Figure 2A (11.6 Hz, compared to 29.3 Hz in Figure 3A), while the amide peak is relatively more intense. Our recent ^1H NMR work on plasma[26] has demonstrated that resonances due to lipoproteins are affected by fasting as well as freezing and thawing of plasma, suggesting that the peak at −334 ppm is a composite peak for the various lipoproteins.

NH_3 release from cisplatin was observed both in blood plasma supplemented with L-Met and in cisplatin-treated plasma with added DEDTC. In neither case were resonances for any Pt(II)-containing species observed by ^{14}N NMR spectroscopy. Cisplatin is known to bind to plasma proteins.[8] It is not possible to say from the ^{14}N studies alone whether NH_3 release occurs as a result of binding to L-Met or to proteins. Our ^1H NMR studies show that in the plasma ultrafiltrates (10-kDa cutoff), with most of the protein removed, both *cis*- and *trans*-$PtCl_2(NH_3)_2$ react with L-Met, while in blood plasma cisplatin reacts with L-Met, but *trans*-$PtCl_2(NH_3)_2$ shows no discernible reaction even after incubation at 37 °C for 28 h.

The resonances of the released ammonium ion in plasma are relatively broad. This broadening may result from the binding of NH_4^+ to proteins. Balaban and Knepper[16a] noted that addition of albumin to aqueous solutions of urea resulted in broadening of the ^{14}N NMR signal for urea and attributed this to a binding process. Similarly, when we added sodium nitrate (1 mM) to plasma, the nitrate resonance was broadened beyond detection.

Clinical Relevance. Because of the current sensitivity limits of ^{14}N NMR, it was possible to study platinum diamines only at concentrations down to ca. 0.4 mM with reasonable accumulation times (up to 12 h). We therefore supplemented the plasma with 0.2 mM L-Met. Typical Pt concentrations in plasma of cisplatin-treated patients[8c] are ca. 7 μM, and biological methionine levels[27] are ca. 35 μM, although the latter may well increase to higher levels in disease states.[28]

DEDTC is given to patients undergoing cisplatin therapy to protect them from toxic side effects.[11] The conditions used in our study (Figure 3) are relevant to cisplatin overdose with "rescue" by the addition of NaDEDTC: elevated levels of cisplatin compared to those found clinically and a 10-fold excess of DEDTC.[11] Under these conditions, there is considerable production of ammonium ions, which can itself lead to toxic effects. Normal NH_4^+ levels in plasma range[29] from 18 to 72 μM. We were able to detect NH_4^+ by ^{14}N NMR spectroscopy at levels around 0.4 mM, and it may be possible to improve on this significantly by the use of higher frequencies and larger sample tubes.

Conclusion

We conclude that ^{14}N NMR spectroscopy can provide useful information about amine release from *cis*-$PtCl_2(NH_3)_2$ and related complexes under biologically relevant conditions. Such NMR studies illustrate that it is now possible to study the coordination chemistry of metal complexes directly in biological media. They may enhance our understanding of the biological chemistry of cisplatin both in vitro and in vivo and be applicable to a range of other metal complexes.

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Registry No. L-Met, 63-68-3; *N*-Ac-L-Met, 65-82-7; $PtCl_2(en)$, 14096-51-6; cisplatin, 15663-27-1.

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