Release of Platinum from Cysteine Residues Induced by N,S-Donor Chelation

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Received January 7, 1992

A limitation in the use of cisplatin as an antitumor drug is its concentration-dependent nephrotoxicity.[1,2] Agents such as sodium diethyldithiocarbamate, [Na][ddtc], have been found which reduce this toxicity. Unfortunately, these agents cause severe side effects such as the burning of the mouth, chest tightness, and extreme anxiety caused by [Na][ddtc].[3] Nephrotoxicity is believed[4] to result from the binding of platinum to sulfur-functionalized protein residues. This theory is supported by the finding that [Na][ddtc] is highly effective in the release of platinum from α_2-macroglobulin.[5] A recent modeling study[6] has indicated that ddtc and other reagents which reduce cisplatin nephrotoxicity only release platinum bound to the thioether sulfur of methionine residues and are completely ineffective in removing platinum from the thiolato sulfur of cysteine residues. This study is in agreement with the finding[5] that platinum binds to α_2-macroglobulin through a methionine site. Thus there is a need to develop improved protein rescue agents which are free of side effects and will release platinum from protein cysteinato sites.

We have prepared diplatinum bis(phosphine) complexes of *N*-acetylcysteine and glutathione. Unlike cysteine residue platinum complexes containing highly labile ammine ligands, these complexes are not subject to rapid decomposition through Pt–S polymerization[7] and are thus free of complications connected with modeling studies based on the ammine complexes. We report here our finding that the platinum thiolato bonds of our diplatinum complexes are remarkably susceptible to cleavage through reaction with free cysteine. The recently found[8] antitumor activity of bis(phosphino)platinum complexes adds to the relevance of the study of phosphinoplatinum–cysteine residue complexes.

Reaction of $PtCl_2(PMe_3)_2$ (**1**) with 1 equiv of *N*-acetylcysteine (Haccys) or glutathione (HGS) in methanol for 24 h results in the formation of $[Pt_2(\mu_S\text{-accys})_2(PMe_3)_4]^{2+}$ (**2**) and $[Pt_2(\mu_S\text{-GS})_2(PMe_3)_4]^{2+}$ (**3**), as seen in Scheme I. Alternatively, **2** and **3** can be obtained through the reaction of $[Pt_2(\mu\text{-OH})_2(PMe_3)_4][NO_3]_2$[9] (**4**) with 2 equiv of Haccys or HGS in distilled water for 2 h. In both cases purified products are obtained in >85% yield. The closely related ammine complexes $[Pt_2(\mu_S\text{-accys})_2(NH_3)_4][NO_3]_2$ and $[Pt_2(\mu_S\text{-GS})_2(NH_3)_4][NO_3]_2$ were recently generated in solution by Appleton et al. and characterized by multinuclear NMR spectroscopic studies[7] which were indicative of diplatinum bis(μ_S-thiolato) formulations. Similarly, the presence of cis diastereotopic PMe_3 ligands in **2** and **3** results in the second-order appearance of the signals for these groups in the $^{13}C\{^1H\}$ and ^1H NMR spectra of the complexes.

By contrast, reaction of cysteine-free base with **1** or **4** gives rise to $[Pt\{\eta_{NS}^2\text{-}SCH_2CH[C(O)OH](NH_2)\}(PMe_3)_2]^{1+}$ (**5**), as seen in Scheme I. Purified **5** is obtained from both reactions >80% yield following recrystallization from methanol/diethyl ether. The mononuclear, η_{NS}^2-cysteinato formulation of **5** is clearly indicated by the appearance of the signals for the PMe_3 ligands as two clean doublets with ^{195}Pt satellites in both the ^1H and $^{31}P\{^1H\}$ NMR spectra of the complex. Formation of the similar monoplatinum cysteine chelate complex, $[Pt\{\eta_{NS}^2\text{-}SCH_2CH[C(O)OH](NH_2)\}(bpy)][Cl]$ was previously found[10] to result from the reaction of $PtCl_2(bpy)$ with $[Na][SCH_2CH[C(O)OH](NH_2)]$. Apparently, coordination of the cysteine amino nitrogen to generate the five-membered, N,S-metallacycles is thermodynamically preferred over formation of dimetallic bis(μ_S-thiolato) complexes.

Cysteine has been found[5] to have some activity in releasing platinum from α_2-macroglobulin even at the low concentrations required to prevent cleavage of protein disulfide bonds. Our results suggested that release of platinum from our μ_S-cysteine residue complexes might be induced by their reaction with free cysteine to form the N,S-metallacycle complex, **5**. In order to explore this possibility, cysteine was reacted with **2** and **3** at pH 7.0 in distilled water at 25 °C. As seen in Scheme I, we find complete conversion of both **2** and **3** to **5** within 4 min. Although it has not been established whether platinum binding to protein cysteine residues occurs through μ_S-thiolato interactions, clearly formation of the N,S-metallacycle provides a sufficient thermodynamic driving force to induce the release of platinum from thiolato ligands. This finding stands in contrast to the modeling studies in which [Na][ddtc] was found only to release platinum bound to the thioether residues and was completely ineffective in removing platinum from the thiolato sulfur.[7] The kinetics of the platinum release reaction observed for our μ_S-cysteine residue complexes are undoubtedly enhanced by the much greater trans labilizing effect of PMe_3 vs NH_3. However, the present study suggests that agents which lead to the formation of N,S-metallacycles may be effective in releasing platinum from protein cysteinato sites.

This work points to the potential use of N,S-donor ligands capable of forming five-membered metallacycles to induce release of platinum from protein cysteine residues. In order to further investigate this strategy for reversing the nephrotoxicity associated with platinum antitumor drugs, we are currently extending these studies to other model platinum complexes containing chelating amines.

Experimental Section

Apparatus and Materials. The following were purchased from Aldrich Chemical Co. and used without further purification: *N*-acetyl-L-cysteine, L-cysteine, and glutathione. The complex $[Pt(PMe_3)_2(\mu\text{-OH})]_2(NO_3)_2$ was prepared by the literature method.[9] The ^1H NMR spectra were recorded on a QE-300 spectrometer at 300 MHz, the ^{13}C NMR spectra were recorded on a GN Omega 500 spectrometer at 125.8 MHz, and the ^{31}P spectra were recorded on a Nicolet NT 300 spectrometer at 122 MHz. The ^1H NMR data are listed in ppm relative to the H_2O signal in D_2O at 4.67 ppm. The ^{31}P NMR chemical shifts were measured relative to the deuterium resonance of the solvent by using the internal frequency lock of the spectrometer so that the resonance in a 5-mm NMR tube containing the deuterated solvent (D_2O) appeared at 0.00 ppm at 20 °C. Elemental analyses were performed by Oneida Research Services Inc. (Whitesboro, NY).

Preparation of $[Pt(PMe_3)_2(\mu_S\text{-accys})]_2[NO_3]_2$, [2][NO$_3$]$_2$. A solution of $[Pt(PMe_3)_2(\mu\text{-OH})]_2(NO_3)_2$ (0.102 g, 239 μmol) in H_2O (30 mL) was treated with *N*-acetylcysteine (0.096 g, 0.239 mmol) and allowed to stir for 2 h. The solvent was then removed under reduced pressure, and the

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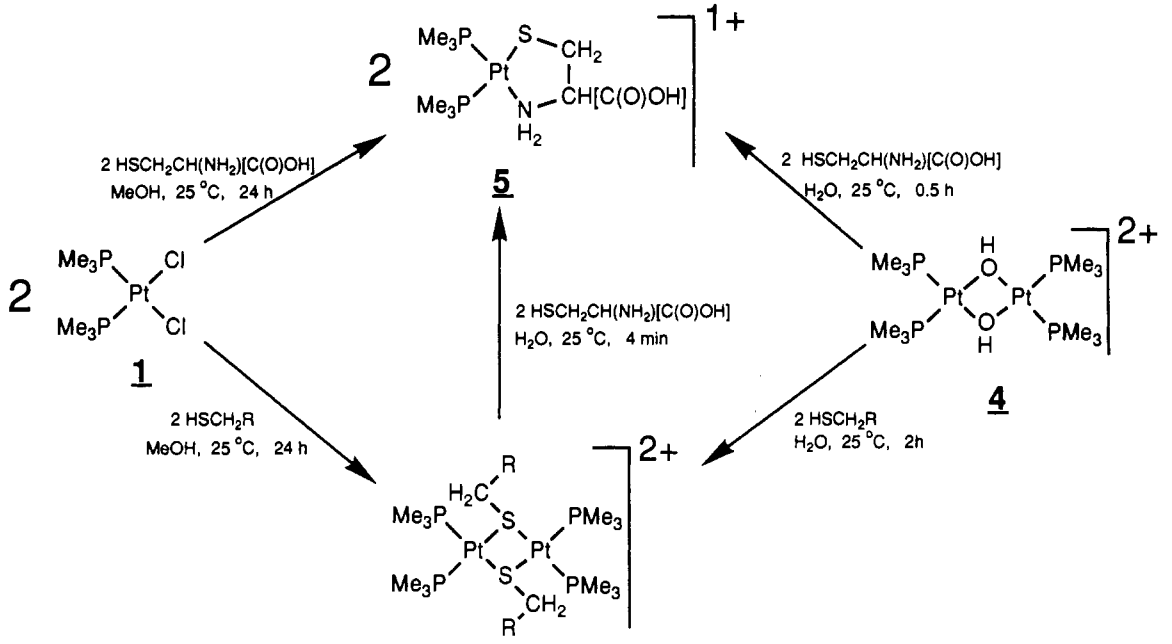
0020-1669/92/1331-3867$03.00/0

Scheme I



R = CH[NHC(O)CH3[C(O)OH] **2**
CH[C(O)NHCH2C(O)OH][NHC(O)C2H4CH(NH2)(C(O)OH)] **3**

resulting light yellow oily residue was dried overnight on a vacuum line to remove all excess H_2O. The resulting amorphous, hygroscopic solid was recrystallized under nitrogen from methanol/diethyl ether. White, microcrystalline [**2**][NO_3]$_2$ (0.296 g, 88% yield) was then recovered upon filtration of the solution under nitrogen. ^{1}H NMR (D_2O), δ: 5.41 (br m, CH_2CH(NHC(O)OMe)(C(O)OH)); 3.65, 3.23 (br m, SCH_2); 2.21 (s, $COCH_3$); 1.81 (m, $P(CH_3)_3$). ^{31}P{^{1}H} NMR (D_2O), δ: −16.3 (s, J_{Pt-P} = 2843 Hz). ^{13}C{^{1}H} NMR (D_2O), δ: 173.8 (s, COOH), 172.7 (s, COMe), 56.5 (s, CH_2CH(NHC(O)OMe)(C(O)OH)), 36.5 (s, SCH_2), 22.2 (s, $COCH_3$), 13.7 (m, PMe_3). Anal. Calcd for [**2**][NO_3]$_2$·H_2O: C, 22.76; H, 4.70; N, 4.82. Found: C, 22.40; H, 4.32; N, 4.54.

Preparation of [Pt(PMe$_3$)$_2$(μ_S-accys)]$_2$[Cl]$_2$, [2][Cl]$_2$. A solution of $PtCl_2(PMe_3)_2$ (**1**) (0.100 g, 0.239 mmol) in methanol (40 mL) was treated with *N*-acetylcysteine (0.039 g, 239 μmol) and allowed to stir for 24 h. The solvent volume was then reduced to ca. 5 mL by vacuum. The product was then precipitated from solution upon addition of diethyl ether. White, microcrystalline [**2**][Cl]$_2$ (0.108 g, 84% yield) was then recovered upon filtration of the solution under nitrogen. ^{1}H, ^{31}P{^{1}H}, and ^{13}C{^{1}H} NMR spectra identical to those obtained for [**2**][NO_3]$_2$ were recorded for a sample of the product in D_2O solution.

Preparation of [Pt(PMe$_3$)$_2$(μ_S-GS)]$_2$[NO$_3$]$_2$, [3][NO$_3$]$_2$. The complex was prepared and isolated (0.288 g, 86% yield) by substitution of glutathione (0.074 g, 239 μmol) into the preparation of [**2**][NO_3]$_2$. ^{1}H NMR (D_2O), δ: 5.10 (m, CH_2CH(NH_2)(C(O)OH)); 4.09 (m, $NHCH$C(O)CH_2); 4.06 (s, $NHCH_2$(C(O)OH)); 3.68, 3.34 (m, SCH_2); 2.70 (m, CH_2CH_2C(O)); 2.28 (m, (NH_2)(C(O)OH)$CHCH_2CH_2$); 1.79 (m, $P(CH_3)_3$). ^{31}P{^{1}H} NMR (D_2O), δ: −17.3 (s, J_{Pt-P} = 2877 Hz). ^{13}C{^{1}H} NMR (D_2O), δ: 174.1 (s, COOH), 172.7 (s, COOH); 171.7 (s, NCO), 171.1 (s, NCO), 56.5 (s, CH_2CH(NH_2)(C(O)OH)), 52.3 (s, SCH_2CH), 41.4 (s, $NHCH_2$(C(O)OH)), 35.3 (s, SCH_2), 31.6 (CH_2CH_2C(O)), 25.6 (s, CH_2CH_2CH), 14.3 (m, PMe_3).

Preparation of [Pt(PMe$_3$)$_2$(μ_S-GS)]$_2$[Cl]$_2$, [3][Cl]$_2$. The complex was prepared and isolated (0.152 g, 92% yield) by substitution of glutathione (0.074 g, 239 μmol) into the preparation of [**2**][Cl]$_2$. ^{1}H, ^{31}P{^{1}H}, and ^{13}C{^{1}H} NMR spectra identical to those obtained for [**3**][NO_3]$_2$ were recorded for a sample of the product in D_2O solution.

Preparation of [Pt(PMe$_3$)$_2${η_{NS}^2-SCH$_2$CH[C(O)OH](NH$_2$)}][NO$_3$], [5][NO$_3$]. A solution of [Pt(PMe_3)$_2$(μ-OH)]$_2$(NO_3)$_2$ (0.102 g, 239 μmol) in H_2O (25 mL) was treated with cysteine-free base (0.029 g, 239 μmol). The resulting solution was stirred for 0.5 h. The solvent was then reduced under reduced pressure, and the resulting light yellow oily residue was dried overnight on vacuum line to remove all excess H_2O. The resulting amorphous, hygroscopic solid was recrystallized under nitrogen from methanol/diethyl ether. White, microcrystalline [**5**][NO_3]$_2$ (0.256 g, 83% yield) was then recovered upon filtration of the solution under nitrogen. ^{1}H NMR (D_2O), δ: 4.03 (m, CH, J_{Pt-H} = 50 Hz, 1 H), 2.75 (m, CH_2, 2 H), 1.63 (m, $P(CH_3)_3$). ^{31}P{^{1}H} NMR (D_2O), δ: −22.1 (d, J_{P-P} = 25 Hz, J_{Pt-P} = 2686 Hz), −32.1 (d, J_{P-P} = 25 Hz, J_{Pt-P} = 3128 Hz). ^{13}C{^{1}H} NMR (D_2O), δ: 173.5 (s, COOH), 65.4 (s, CH), 29.8 (s, CH_2), 15.5 (d, $P(CH_3)_3$, J_{P-C} = 45 Hz, J_{Pt-C} = 40 Hz), 14.7 (d, $P(CH_3)_3$, J_{P-C} = 39 Hz, J_{Pt-C} = 30 Hz). Anal. Calcd for [**5**][NO_3]·H_2O: C, 19.74; H, 4.79; N, 5.12. Found: C, 19.53; H, 4.38; N, 4.96.

Preparation of [Pt(PMe$_3$)$_2${η_{NS}^2-SCH$_2$CH[C(O)OH](NH$_2$)}][Cl], [5][Cl]. A solution of $PtCl_2(PMe_3)_2$ (0.100 g, 239 μmol) in methanol (40 mL) was treated with cysteine-free base (0.029 g, 239 μmol). The solution was stirred for 24 h. The solvent volume was then reduced to ca. 5 mL by vacuum. The product was then precipitated from solution upon addition of diethyl ether. White solid [**5**][Cl]$_2$ (0.116 g, 97% yield) was then recovered upon filtration of the solution under nitrogen in the drybox. ^{1}H, ^{31}P{^{1}H}, and ^{13}C{^{1}H} NMR spectra identical to those obtained for [**5**][NO_3]$_2$ were recorded for a sample of the product in D_2O solution.

Reactions of [2] and [3] with Cysteine. A solution of [**2**][Cl]$_2$ (0.260 g, 239 μmol) in distilled water (40 mL) was brought to pH 7.0 with potassium phosphate monobasic–sodium hydroxide buffer solution and treated with cysteine (0.029 g, 239 μmol). The solvent was immediately removed under reduced pressure. The ^{31}P NMR spectrum of the resulting white residue was identical to that recorded for [**5**][Cl]. An analogous experiment carried out with [**3**][Cl]$_2$ (0.329 g, 239 μmol) was also seen to produce [**5**][Cl].

A solution of [**2**][Cl]$_2$ (0.027 g, 25 μmol) in D_2O (0.3 mL) was treated with cysteine (0.003 g, 25 μmol). The ^{31}P NMR spectrum of the sample was obtained within 4 min of mixing and indicated that complete conversion to [**5**][Cl] had occurred.

Acknowledgment. We thank Mr. Wesley Yoshida for his assistance with the NMR spectroscopic studies. This work was supported by the University of Hawaii Research Council.