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Reaction of platinum(II) diamine and triamine complexes with selenomethionine

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ABSTRACT

We have reacted [Pt(dien)Cl]Cl, $[Pt(en)(D_2O)_2]^{2+}$, and $[Pt(Me_4en)(D_2O)_2]^{2+}$ [Me_4en = *N,N,N′,N′*-tetramethylethylenediamine] with selenomethionine (SeMet). When [Pt(dien)Cl]Cl is reacted with SeMet, $[Pt(dien)(SeMet\text{-}Se)]^{2+}$ is formed; two Se–CH_3 resonances are observed due to the different chiralities at the Se atom upon platination. In a reaction of [Pt(dien)Cl]Cl with an equimolar mixture of SeMet and Met, the SeMet product forms more quickly though a slow equilibrium with approximately equal amounts of both products is reached. $[Pt(Me_4en)(D_2O)_2]^{2+}$ reacts with SeMet to form $[Pt(Me_4en)(SeMet\text{-}Se)(D_2O)]^{2+}$ initially but forms $[Pt(Me_4en)(SeMet\text{-}Se,N)]^{+}$ ultimately. One stereoisomer of the chelate, assigned to the R chirality at the Se atom, dominates within the first few minutes of reaction. $[Pt(en)(D_2O)_2]^{2+}$ forms a variety of products depending on reaction stoichiometry; when one equivalent or less of SeMet is added, the dominant product is $[Pt(en)(SeMet\text{-}Se,N)]^{+}$. In the presence of excess SeMet, $[Pt(en)(SeMet\text{-}Se)_2]^{2+}$ is the dominant initially, but displacement of the en ligand occurs leading to $[Pt(SeMet\text{-}Se,N)_2]$ as the eventual product. Displacement of the en ligand from $[Pt(en)(SeMet\text{-}Se,N)]^{+}$ does not occur. In reactions of K_2PtCl_4 with two equivalents of SeMet, $[Pt(SeMet\text{-}Se,N)_2]$ is formed, and three sets of resonances are observed due to different chiralities at the Se atoms. Only the *cis* geometric isomers are observed by ^1H and ^{195}Pt NMR spectroscopy.

1. Introduction

Complexes of the type *cis*-PtA_2X_2, where A_2 represents two unidentate or one bidentate amine ligand and X_2 represents two monodentate or one bidentate leaving group, have been widely studied in part due to the anticancer activity of cisplatin, *cis*-$Pt(NH_3)_2Cl_2$. Cisplatin's anticancer activity has been attributed to the formation of a 1,2-intrastrand crosslink between two adjacent guanine residues in DNA [1]. However, interaction with proteins may be important in transport of platinum complexes into the cell [2–4]. Furthermore, [Pt(dien)Cl]Cl has been shown to react faster with methionine analogs than with 5′-GMP [5,6]. Thus, a better understanding of the interaction of platinum complexes with amino acids is necessary.

Platinum complexes have a high affinity for methionine, a standard amino acid found in proteins. Depending on factors such as pH, stoichiometry, and the bulk of the amine ligands on the platinum, a variety of products result from the reaction of methionine or *N*-acetylmethionine with *cis*-PtA_2X_2 complexes. When methionine is present in excess, complexes of the type *cis*-PtA_2(Met-*S*)$_2$ may be observed [7]. Chelates in which the sulfur atom and either a carboxyl oxygen or the amine nitrogen are coordinated to the platinum are also known [7–9]. The *trans* effect of the methionine sulfur atom can lead to displacement of the ammine ligands of cisplatin [10,11] and can sometimes lead to displacement of a nitrogen atom from a chelated diamine such as ethylenediamine (en) [8,12].

Selenomethionine (SeMet) is a nonstandard amino acid in which the sulfur atom of methionine is replaced by selenium. Unlike selenocysteine, which is intentionally inserted into proteins such as glutathione peroxidase, selenomethionine may be randomly inserted into proteins in place of methionine [13]. In addition to the presence of nonstandard selenium-containing amino acids, some organoselenium compounds have been in clinical trials as cancer chemopreventative drugs. Selenomethionine was found to have protective effects against cisplatin-induced toxicity in rats and mice [14]. Both 5-methylselenocysteine and selenomethionine greatly increased survival in nude mice that were administered toxic doses of cisplatin and oxaliplatin [15]. Thus, previous studies have focused on the molecular interaction of cisplatin and analogs with selenomethionine [16,17].

Selenium, like sulfur, has a significant *trans* effect that can lead to rapid displacement of an ammine ligand of cisplatin. Mass spec-

Abbreviations: SeMet, selenomethionine; N-AcMet, N-acetylmethionine.

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trometry studies of the reaction between cisplatin and selenomethionine found that even at 1:1 SeMet:Pt ratios, displacement of one ammine ligand occurs to a significant amount within 10 min [17]. For both cisplatin and carboplatin, a significant amount of $[Pt(SeMet\text{-}Se,N)_2]^+$ was detected by mass spectrometry within 24 h [16,17], indicating that both ammine ligands had been displaced.

In this study, we have utilized platinum complexes that contain chelated diamine and triamine ligands. We wanted to use these platinum complexes to characterize SeMet complexes that would be less prone to amine ligand displacement than the cisplatin analogs used previously [16,17]. We also wanted to determine whether Met or SeMet would react preferentially with platinum complexes.

2. Experimental

K_2PtCl_4, diethylenetriamine, $Pt(en)Cl_2$, selenomethionine, methionine, and silver nitrate were used as received. [Pt(dien)Cl]Cl and $Pt(Me_4en)Cl_2$ were prepared by methods described previously [9,18].

$Pt(en)(NO_3)_2$ and $Pt(Me_4en)(NO_3)_2$ were prepared by reacting two equivalents of $AgNO_3$ with the appropriate platinum(II) dichloride compound in H_2O and stirring in an amber vial overnight. The samples were filtered to remove AgCl precipitate and evaporated to dryness. When added to D_2O, $[Pt(en)(D_2O)_2]^{2+}$ and $[Pt(Me_4en)(D_2O)_2]^{2+}$ would be expected as the dominant species due to the very small association constant reported for NO_3 with platinum complexes [19].

^1H and ^{195}Pt NMR spectra were acquired on a JEOL Eclipse 500 MHz NMR instrument. The ^1H NMR spectra were referenced to the residual HOD signal relative to TSP, adjusted for temperature. The ^{195}Pt NMR spectra were referenced relative to K_2PtCl_6. All spectra were collected at room temperature unless otherwise noted.

A Hitachi LaChrom Elite HPLC system with a cation-exchange column was utilized. Buffer A was 20 mM sodium phosphate at pH 6, while buffer B was 20 mM sodium phosphate with 0.5 M NaCl added at pH 6. The gradient was as follows: $t = 0$, 100% A; $t = 2$ min, 100% A; $t = 20$ min, 50% A.

Molecular mechanics calculations were performed according to methods described previously using a modified AMBER force field [9].

3. Results

3.1. Reaction of [Pt(dien)Cl]Cl with SeMet

[Pt(dien)Cl]Cl and SeMet (10 mM each) were combined in D_2O at pH 5. A ^1H NMR spectrum acquired ~45 min later showed new resonances; two singlets at 2.41 and 2.42 ppm were observed;

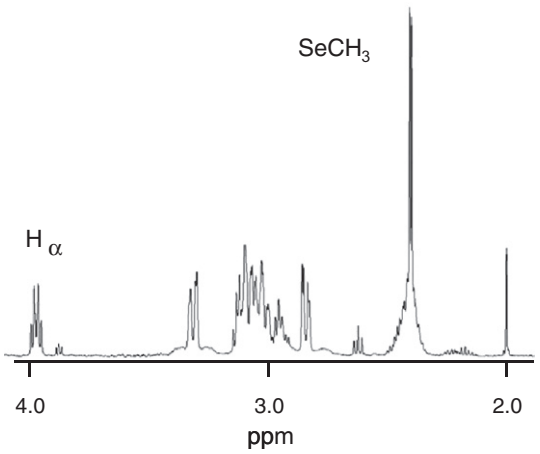

Fig. 1. Partial ^1H NMR spectrum of the reaction of [Pt(dien)Cl]Cl with SeMet at pH ~4.5 at room temperature. Initial concentrations of both are 10 mM.

these singlets were ~0.4 ppm downfield of the singlet observed for unreacted SeMet (Fig. 1). A downfield shift of similar magnitude has been previously observed for *S*-CH_3 resonances of Met upon coordination of the Met to platinum via the sulfur atom [9,20,21], and similar shifts were observed with previous complexes of platinum with selenomethionine [16,17]. Thus, the singlets were assigned to the CH_3 resonance of $[Pt(dien)(SeMet\text{-}Se)]^{2+}$. A COSY spectrum was used to assign the additional product resonances (Table 1).

The presence of two CH_3 resonances suggests that relatively slow interconversion of chirality about the Se atom is occurring. Only one CH_3 resonance was observed previously for the analogous $[Pt(dien)(Met\text{-}S)]^{2+}$ complex [5]; however, the presence of two sets of resonances has been observed previously for a number of platinum complexes with sulfur-coordinated methionine by ^1H and/or ^{195}Pt NMR methods [9,22,23] and multiple sets of resonances were observed in a [^1H, ^{15}N] HSQC NMR spectrum of a product formed between carboplatin and SeMet [16]. Support for the assignment of two interconverting chiralities came from variable temperature experiments that showed the signals broadening by 60 °C and beginning to coalesce by 80 °C. A single peak at −3420 ppm was observed in the ^{195}Pt NMR spectrum at room temperature.

3.2. Reaction of [Pt(dien)Cl]Cl with SeMet and Met

In order to determine the relative reactivities of SeMet and Met with a selected platinum complex, [Pt(dien)Cl]Cl was added to a mixture of SeMet and Met to give a solution that was 5 mM in all three components. During the first several hours of reaction, the ^1H NMR spectrum showed signals corresponding to $[Pt(dien)(SeMet\text{-}Se)]^{2+}$ and $[Pt(dien)(Met\text{-}S)]^{2+}$; however, the signals of the

Table 1
^1H and ^{195}Pt NMR chemical shifts of SeMet complexes.

	CH_3	H_α	H_β	H_γ	^{195}Pt
SeMet	2.02	3.84	2.19, 2.24	2.63	N/A
$[Pt(dien)(SeMet\text{-}Se)]^{2+}$	2.44, 2.43	3.96	2.44	3.09, 2.98	−3420
$[Pt(Me_4en)(SeMet\text{-}Se,N)]^+$R	2.49	3.55	2.36, 2.79	2.91	−3190
$[Pt(Me_4en)(SeMet\text{-}Se,N)]^+$S	2.47	3.55	2.14, 2.87	3.17	−3260
$[Pt(en)(SeMet\text{-}Se,N)]^+$	2.41, 2.43	3.48	2.32, 2.54	3.05, 2.93	−3381, −3393
$[Pt(en)(SeMet\text{-}Se)_2]^{2+}$	2.53, 2.54	3.86	2.25, 2.39	3.07, 3.18	−3910
cis-$[Pt(SeMet\text{-}Se,N)_2]$ RR	2.55	3.73	2.36, 2.68	2.97, 3.04	−3882
cis-$[Pt(SeMet\text{-}Se,N)_2]$ RS	2.50, 2.46	3.70, 3.76	2.28, 2.52[a]	[a]	−3837
cis-$[Pt(SeMet\text{-}Se,N)_2]$ SS	2.49	3.85	2.20, 2.44	3.09[a]	−3815

[a] Incomplete assignment due to overlap with other signals.