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Interaction of *N*-acetylmethionine with a non-C_2-symmetrical platinum diamine complex

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Abstract

The reaction of *N*-acetylmethionine (*N*-AcMet) with the complex $[Pt(Et_2en)(D_2O)_2]^{2+}$ ($Et_2en = N,N$-diethylethylenediamine) was studied by NMR spectroscopy and molecular mechanics calculations. Complexes containing two methionine residues coordinated to the platinum atom were calculated to be relatively high in energy unless the bulk of the methionine residues was directed away from the diethyl group of the Et_2en ligand. In contrast, sulfur–oxygen chelates were found to be relatively free of steric clashes. Experimentally, two sets of NMR resonances were observed when $[Pt(Et_2en)(D_2O)_2]^{2+}$ was reacted with *N*-AcMet; variable temperature experiments indicated intermediate chemical exchange between the two sets of resonances. NMR studies indicated that the resonances corresponded to $[Pt(Et_2en)(N\text{-AcMet-}S,O)]^+$ complexes with the sulfur atom trans to the diethyl group of the Et_2en ligand. No product with the sulfur atom cis to the diethyl group was observed experimentally even though molecular mechanics calculations suggested that such forms have few steric clashes. The NMR results suggested that the chemical exchange was a result of sulfur chirality inversion. In early stages of the reaction, a $[Pt(Et_2en)(N\text{-AcMet-}S)(D_2O)]^+$ complex was observed, indicating that coordination of the oxygen to form the chelate is relatively slow.

Keywords: Nuclear magnetic resonance; Molecular modeling; Ligand binding; Anticancer drug

1. Introduction

Platinum diamine complexes are known to interact with both DNA and protein targets. The interaction of certain platinum diamine complexes with DNA has been widely studied due to the anticancer activity of compounds such as cisplatin (*cis*-$Pt(NH_3)_2Cl_2$) [1,2]. Cisplatin reacts primarily with the *N*7 atom of guanine residues in DNA, and a 1,2-intrastrand cross-link between adjacent guanines leads to a distortion of the double helix.

The reaction of platinum complexes with proteins is a competing reaction that may result in toxic side effects or the development of resistance in anticancer compounds [3,4]. Platinum has a high affinity for the sulfur atoms of both methionine and cysteine residues; however, cysteine residues are often involved in disulfide bonds, which are not good ligands for platinum [5]. Reaction of platinum complexes with methionine is also important because the complex $[Pt(en)(H_2O)_2]^{2+}$ [en = ethylenediamine] has been utilized to selectively cleave proteins at the C-terminal side of methionine; coordination to the sulfur atom of methionine and interaction with the oxygen atom of the C-terminal amide appear to be important for the cleavage reaction [5,6]. Therefore, it is important to gain a better understanding of the factors affecting the interaction of platinum complexes with methionine.

Platinum complexes containing a relatively small diamine ligand such as en can react with an excess of

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a methionine derivative to form complexes of the type $[Pt(en)(Met\text{-}S)_2]^{2+}$ and $[Pt(en)(N\text{-}AcMet\text{-}S)_2]$ [Met = methionine, *N*-AcMet = *N*-acetylmethionine] [7,8]. In the presence of a 1:1 ratio of platinum to methionine, the chelate [Pt(en)(*N*-AcMet-*S*,*N*)] was observed [8] in aqueous solution. Analogous *S*,*N* chelates have been observed in many studies involving platinum complexes coordinated to peptides [9,10].

Previously, we have shown that the complex $[Pt(Me_4en)(D_2O)_2]^{2+}$ [Me_4en = *N*,*N*,*N*′,*N*′-tetramethylethylenediamine] (Fig. 1), which contains a bulky diamine ligand, reacts with methionine and *N*-acetylmethionine to form only a 1:1 complex even when the methionine ligand is present in excess [7]. Molecular mechanics calculations indicated that a $[Pt(Me_4en)(Met\text{-}S)_2]^{2+}$ complex would have severe steric clashes. NMR results indicated that $[Pt(Me_4en)(N\text{-}AcMet\text{-}S,O)]^{+}$ and $[Pt(Me_4en)(Met\text{-}S,N)]^{+}$ chelates were observed with *N*-acetylmethionine and methionine, respectively. Molecular mechanics calculations suggested that the presence of the *N*-acetyl group in the former prevented coordination of the amide nitrogen to the platinum atom.

In the present study, we have utilized platinum complexes containing the non-C_2-symmetrical Et_2en ligand [Et_2en = *N*,*N*-diethylethylenediamine] (Fig. 1). We have utilized a modified AMBER force field to examine structures of various methionine complexes that could form. Experimentally, we have utilized both ^1H and ^{195}Pt NMR spectroscopy to characterize the complexes that are observed in the reaction of $[Pt(Et_2en)(D_2O)_2]^{2+}$ with *N*-acetylmethionine. We have utilized the Et_2en ligand in order to determine whether significant bulk on only one of the two nitrogens of the diamine ligand is sufficient to prevent coordination of a second methionine residue or to influence the type of chelate that forms. Furthermore, because the Et_2en ligand is non-C_2-symmetrical, reaction of one *N*-acetylmethionine with the platinum complex could lead to two geometrical isomers in which the sulfur atom is either *cis* or *trans* to the nitrogen atom with the two ethyl groups. The relative amounts of the geometrical isomers could lead to insights regarding the effect of bulk on the kinetics and/or thermodynamics of complex formation. Finally, the presence of two hydrogen atoms on one of the nitrogen atoms could result in different kinetics of reaction compared to complexes with the Me_4en ligand.

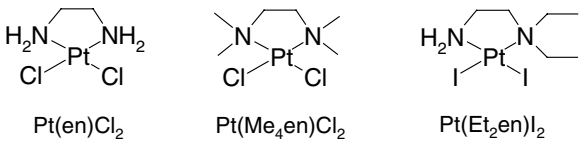

Fig. 1. Representations of platinum compounds with varying amounts of bulk at the amine nitrogens.

2. Materials and methods

Commercial reagent grade chemicals were utilized without further purification.

2.1. Molecular mechanics calculations

Molecular mechanics calculations were performed using HyperChem 7 (Hyper, Inc.) on a Dell Optiplex GX270. The AMBER force field was modified with previous parameters for platinum coordinated to guanine [11] and methionine [7] residues. Charges were set as described previously [7].

The distance-dependent dielectric constant (4**r*) was utilized, and the 1–4 nonbond parameters were scaled by 0.5. Molecular dynamics were typically run at a simulated temperature of 300 K for 250 ps. Structures were saved every 1 ps, and these structures were subjected to 1000 cycles of steepest descents and 10,000 cycles of Fletcher–Reeves conjugate gradient minimizations or until the gradient was <0.01 kcal/mol Å.

2.2. Synthesis of $Pt(Et_2en)I_2$

The $Pt(Et_2en)I_2$ compound was synthesized based on previous methods [12,13]. In a typical synthesis, 220 mg of K_2PtCl_4 was dissolved in 10 mL H_2O and an excess (~4 g) of KI was added. After stirring for 10 min, 80 μL of *N*,*N*-diethylethylenediamine was added and the reaction was stirred overnight. The precipitate was collected by vacuum filtration and washed with water. Yield: 80%. ^1H NMR (acetone-d_6): 1.48 ppm, triplet, 6H (methyl groups); 2.69 ppm, multiplet, 2H (en CH_2 on diethyl side); 2.96 ppm, multiplet, 2H (en CH_2 on NH_2 side); 3.13 ppm, multiplet, 2H (diethyl CH_2); 3.60 ppm, multiplet, 2H (diethyl CH_2); 4.75 ppm, broad, 2H (NH_2 protons). Broad satellite peaks resulting from ^{195}Pt coupling could be observed for the signals at 2.69, 2.96, and 3.13 ppm.

2.3. Reaction with N-AcMet

$Pt(Et_2en)I_2$ was treated with two equivalents of $AgNO_3$ in D_2O and the reaction mixture was stirred in an amber vial overnight. After filtering the sample, one or two molar equivalents of *N*-AcMet was added to the sample and the pH was adjusted to ~4.

2.4. NMR Spectroscopy

^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 [14]. For the ^{195}Pt NMR spectra, K_2PtCl_6 was used as an external reference ($\delta = 0$ ppm), and the following parameters were