Inorganica Chimica Acta 458 (2017) 163–170

Contents lists available at ScienceDirect

Inorganica Chimica Acta

journal homepage: www.elsevier.com/locate/ica

Research paper

Partial displacement of a triamine ligand from a platinum(II) complex after reaction with N-acetylmethionine

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ARTICLE INFO

Article history:
Received 25 August 2016
Received in revised form 10 January 2017
Accepted 11 January 2017
Available online 13 January 2017

Keywords:
Platinum
Methionine
Guanine
Nuclear magnetic resonance
Molecular mechanics
Raman

ABSTRACT

The compound [Pt(Et_2dien)Cl]Cl, where Et_2dien = N,N-diethyldiethylenetriamine, has been synthesized and reacted with N-acetylmethionine (N-AcMet) and guanosine 5′-monophosphate (5′-GMP). Reaction with 5′-GMP at pH 4 leads to formation of [Pt(Et_2dien)(5′-GMP)]$^+$, as expected. Reaction with N-AcMet occurs more rapidly than with 5′-GMP; when the reaction with N-AcMet occurs at low pH values (~2), one set of resonances is observed initially but a second set of resonances appears over several days as the initial set decreases. The initial set of resonances disappears slowly (several days) at pH 2 but disappears much faster (minutes to hours) if the pH is 4 or higher; the initial resonances do not re-appear even if the pH is lowered. Reaction of the [Pt(Et_2dien)Cl]$^+$ with a mixture of N-AcMet and 5′-GMP at pH 4 leads to formation of a unique set of NMR resonances that does not correspond to the resonances of either the 5′-GMP or the N-AcMet products; mass spectrometry identifies a new product corresponding to [Pt(Et_2dien)(N-AcMet)(5′-GMP)], in which the Et_2dien ligand is bidentate. Such a complex is also observed if 5′-GMP is added to a sample of [Pt(Et_2dien)(N-AcMet)]$^+$ that has been kept at low pH, but it is not observed when N-AcMet is added to a sample of [Pt(Et_2dien)(5′-GMP)]$^+$. We propose that the additional set of resonances observed for [Pt(Et_2dien)(N-AcMet)]$^+$ at low pH is due to a less stable conformation of the complex and the addition of 5′-GMP when this less stable conformation exists leads to displacement of one of the nitrogen atoms.

1. Introduction

Because of the cytotoxic activity of cisplatin and its derivatives, the reactions of platinum complexes with amino acid and nucleotide targets has been studied for many years. Reaction with DNA, primarily at guanine residues, is widely accepted to be responsible for cytotoxicity; however, reaction with proteins is also important. Methionine is one of the primary amino acid targets; as methionine and cysteine residues are a key part of the copper transporter CTR1 [1], which may be partly responsible for cisplatin uptake [2,3], it is important to understand how platinum complexes react with both methionine and guanine.

Reactions of platinum(II) triamine complexes with N-acetylmethionine (N-AcMet) and guanosine 5′-monophosphate (5′-GMP) have been studied as simple models systems of cisplatin with only one leaving ligand. [Pt(dien)Cl]$^+$ has been shown to react faster with thioethers such as S-methylglutathione and methionine than with 5′-GMP [4,5]. [Pt(dien)Cl]$^+$ has therefore represented a simple model system in which only one biological ligand coordinated to the platinum complex. The faster reactivity of thioethers relative to 5′-GMP of this simple model system is consistent with observations that cisplatin formed more adducts with proteins than with DNA [6].

The sterically hindered [Pt(Me_5dien)(H_2O)]$^{2+}$ complex slowed reaction with N-AcMet more than with 5′-GMP so that the latter was kinetically favored [7]. This was the first report of a platinum(II) triamine complex with a significant kinetic preference for 5′-GMP over N-AcMet. Because the 5′-GMP ligand is planar at the site of Pt reaction (N7) and the N-AcMet is not, the steric hindrance affected the methionine reaction more. Later studies with [Pt(en)(D_2O)$_2$]$^{2+}$ and [Pt(Me_4en)(D_2O)$_2$]$^{2+}$ indicated that the steric hindrance in the latter slowed reaction with N-AcMet by a factor of ~16, indicating that having a tertiary amine nitrogen atom *cis*

Abbreviations: Et_2dien, N,N-diethyldiethylenetriamine; Me5dien, N,N,N′,N′,N″-pentamethyldiethylenetriamine; N-AcMet, N-acetylmethionine.

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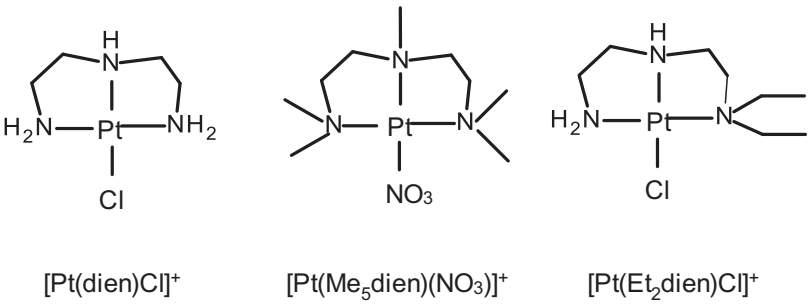
http://dx.doi.org/10.1016/j.ica.2017.01.010
0020-1693/



Scheme 1. Representations of three platinum(II) triamine complexes.

to the coordination site significantly affected the kinetics of the reaction [8].

Separately, reactivity of the highly cytotoxic phenanthriplatin and the less cytotoxic pyriplatin were compared, and the larger size of the ligand of the former was found to slow reaction with N-AcMet more than with 5′-GMP [9]. This observation is consistent with the interpretation from other studies that steric hindrance due to nonleaving ligands has a greater effect on N-AcMet than on 5′-GMP. Unlike the traditional platinum anticancer drugs, phenanthriplatin does not bend the DNA but instead arrests transcriptional machinery. Thus, as the heterocyclic ligands of a series of platinum compounds increased in size, the cytotoxicity increased as well. Other monofunctional compounds with steric hindrance have also shown significant antitumor activity as well [10].

Phenanthriplatin has three nitrogen containing ligands and a single leaving Cl and thus has some similarities to the dien-based triamine complexes; however, only one of the ligands *cis* to the leaving position (the phenanthridine) is sterically hindering, and thus neither the dien nor the Me_5dien ligand have a similar steric pattern to phenanthriplatin though the steric hindrance is on an sp^3 rather than an sp^2 nitrogen atom. The current study focuses on the reactivity of a triamine complex with steric hindrance on only one side of the coordination plane. [Pt(Et_2dien)Cl]Cl, where Et_2dien = N,N-diethyldiethylenetriamine, has been prepared using a method similar to that reported for [Pt(dien)Cl]Cl previously [4]. The Et_2dien ligand has significant steric hindrance on one of the two nitrogen atoms *cis* to the coordination site (see Scheme 1).

Both [Pt(dien)(Met)]$^{2+}$ and [Pt(Me_5dien)(N-AcMet)]$^+$ complexes are prone to slow displacement of the methionine residue when 5′-GMP is added to the solution [5,7]. Thus, the 5′-GMP products are thermodynamically favored even though in the former case the methionine product is kinetically favored. In contrast to the displacement of the methionine residues in these previous studies, the [Pt(Et_2dien)(N-AcMet)]$^+$ complex in the present study shows a unique reactivity with 5′-GMP.

2. Materials and methods

Potassium tetrachloroplatinate (Sigma-Aldrich), N-acetylmethionine (Acros), Guanosine 5′-monophosphate (Sigma-Aldrich), N,N-diethyldiethylenetriamine (Sigma-Aldrich), and 2-hydroxy-4-(methylthio)butyrate (Sigma-Aldrich) were used as received. [Pt(dien)Cl]Cl was synthesized by a previously published procedure [4].

2.1. Synthesis of [Pt(Et_2dien)Cl]Cl

[Pt(Et_2dien)Cl]Cl was synthesized by a method similar to that used for [Pt(dien)Cl]Cl4. Potassium tetrachloroplatinate (1 g) was dissolved in 30 mL of DI water, and 1 mL of N,N-diethyldiethylenetriamine was added. The pH of the solution was lowered to ~3 with HCl, and the solution was refluxed for at least 6 h. The volume of the solution was reduced, and the resulting precipitate was collected and washed with ethanol.

2.2. Reaction of [Pt(Et_2dien)Cl]Cl

Typically, 10 mM stock solutions of [Pt(Et_2dien)Cl]Cl, 5′-GMP, and/or N-AcMet were prepared in D_2O; the pH of each solution was adjusted as necessary. Aliquots of the stock solutions were mixed to give solutions of the appropriate final concentrations.

2.3. NMR spectroscopy

^{1}H and ^{195}Pt NMR data were collected on a JEOL 500 MHz NMR instrument. Spectra were referenced to the residual HOD signal (^{1}H) or K_2PtCl_6 (^{195}Pt).

2.4. Mass spectrometry

Mass spectra were acquired on a Varian LC/MS 500 Ion Trap instrument in positive ion mode using an *m/z* range from 0 to 1100. The samples were injected via direct infusion.

2.5. Molecular mechanics and dynamics calculations

Molecular mechanics and dynamics calculations were performed using HyperChem 7 (Hyper, Inc.) for the PC. The AMBER forcefield with previously described modifications to include platinum bonded to guanine [11] and methionine [12] were included. Charges were set as described previously [12]. Molecular dynamics were run at 300 K for 250 ps, saving structures every 1 ps. The generated structures were subjected to 1000 cycles of steepest descents and 10,000 cycles of Fletcher-Reeves conjugate gradient minimization until the gradient was <0.01 kcal/mol Å.

2.6. Quantum mechanics calculations

The B3LYP density functional method was used with the 6-311G(d,*p*) basis set to optimize the molecular geometries and calculate the vibrational frequencies and Raman intensities using the Gaussian 09 suite of programs. Raman spectra were simulated by summing Lorentzian lineshapes of each frequency.

2.7. Raman spectroscopy

Raman spectra were acquired using a 785 nm laser line and a Horiba Scientific LabRAM HR Evolution Raman Spectroscopy system.