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Effect of Amine Ligand Bulk on the Interaction of Methionine with Platinum(II) Diamine Complexes

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Molecular mechanics and dynamics calculations have been used in conjunction with experimental data to study the effects of amine ligand bulk on the formation of both guanine and methionine complexes with platinum diamine compounds. The AMBER force field has been supplemented with previous modifications [Yao; et al. *Inorg. Chem.* **1994**, *33*, 6061−6077. Cerasino; et al. *Inorg. Chem.* **1997**, *36*, 6070−6079] and has been further modified to include parameters for platinum bound to the sulfur atom of methionine. Molecular mechanics calculations with this modified AMBER force field have suggested that a platinum complex with two sulfur-bound methionine ligands and a bulky diamine ligand (*N*,*N*,*N*′,*N*′-tetramethylethylenediamine, Me_4en) would have severe interligand clashes; such interligand clashes are less pronounced in bis(9-ethylguanine) complexes. Consistent with these observations, NMR studies with $[Pt(Me_4en)(D_2O)_2]^{2+}$ have indicated that guanine 5′-monophosphate reacts in a 2:1 guanine:platinum ratio while both methionine and *N*-acetylmethionine react with only a 1:1 stoichiometry. Methionine forms a chelate via the sulfur and nitrogen atoms whereas *N*-acetylmethionine forms a chelate via the sulfur and oxygen atoms. The oxygen of the latter chelate can be displaced by the addition of guanosine 5′-monophosphate, although complete displacement of the *N*-acetylmethionine was not observed.

Introduction

Cisplatin, *cis*-$Pt(NH_3)_2Cl_2$, is a widely used anticancer drug that can react with both DNA and protein residues.[1−3] Reaction of *cis*-PtA_2Cl_2 complexes (A_2 = two unidentate ligands or one bidentate amine ligand) with DNA occurs predominantly at the guanine *N*7 atom. A 1,2-intrastrand cross-link between adjacent guanine residues causes distortion of the DNA helix and is thought to be primarily responsible for the anticancer activity of the drug.[1,2]

When guanine derivatives are added to solutions of *cis*-PtA_2Cl_2 or $[cis\text{-}PtA_2(D_2O)_2]^{2+}$ (charge varies with pH), coordination of the guanine occurs primarily via the *N*7 atom.[1,2,4−8] At pH 7, 5′-GMP (guanosine 5′-monophosphate) can chelate to the platinum via *N*7 and the phosphate group;[9] however, at a 2:1 GMP:Pt ratio, a bis product with two *N*7-coordinated guanine atoms dominates.[5−8]

Because platinum is a soft metal, the sulfur atoms of methionine and cysteine residues are primary targets in proteins. Protein binding has been suggested to contribute to the toxicity of platinum anticancer drugs;[3] however, it has also been suggested that reaction of platinum with biological thiols may be a pathway for detoxification and resistance.[10] Furthermore, binding of certain *cis*-PtA_2Cl_2 complexes to methionine residues can result in cleavage on the C-terminal side of the residue, providing a convenient method for regioselective chemical cleavage of proteins.[11,12] Thus, a better understanding of the interaction of platinum complexes with both DNA and protein targets is important.

Ligands such as *N*-acetylmethionine (*N*-AcMet), *S*-methylglutathionine, and methionine-containing peptides, all of

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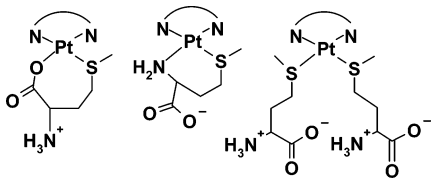

Figure 1. Representations of various complexes formed between platinum diamine compounds and methionine.

which contain a thioether functionality, initially react with *cis*-PtA_2Cl_2 complexes via the sulfur atom (Figure 1).[3,11−16] Chelation via the amide nitrogen is common,[14−16] although two sulfur-coordinated thioether ligands comprise a dominant product when the thioether ligand is small and present in a ∼2:1 ratio relative to the platinum.[14] Additionally, chelates via the sulfur and carboxyl oxygen atom have been observed as a kinetic product.[15]

In competition experiments,[3] *S*-methylglutathione and 5′-GMP were added to a solution of [Pt(dien)Cl]Cl (dien = diethylenetriamine), which contains only one available coordination site. Coordination of platinum to the sulfur atom of the thioether occurred initially, but coordination to the *N*7 atom of 5′-GMP was the eventual thermodynamic product. Analogous experiments utilizing $Pt(en)Cl_2$ (en = ethylenediamine), *N*-AcMet, and 5′-GMP found that 5′-GMP could replace one of two sulfur-coordinated *N*-AcMet ligands.[14]

Many studies have utilized analogues of cisplatin with amine ligands that contain significant steric bulk.[4−8] When *cis*-PtA_2G_2 complexes (G = guanine derivative) with bulky amine ligands are prepared, restricted rotation of the guanine around the Pt−*N*7 bond is typical.[1,4−6] Depending on the chiralities of the amine ligands and of the guanine derivative, one or two head-to-tail (HT) and one or two head-to-head (HH) rotamers are distinguishable, in which an HH rotamer has the *H*8 atoms of the guanines on the same side of the platinum coordination plane.[5]

The presence of chiral amine ligands usually results in a preference for certain rotamers in *cis*-PtA_2G_2 complexes.[5−8] Often the favored rotamers have no ability to form hydrogen bonds between the hydrogen atoms of the coordinated amines and the guanine bases,[6] suggesting that the preference may be primarily due to steric clashes in the disfavored rotamers. In support of this suggestion, the *O*6 atoms of coordinated guanines have been predicted by molecular mechanics (MM) calculations to have steric clashes with alkyl groups on certain amine ligands.[7,8] Thus, it is apparent that interligand clashes between the amine ligand and a coordinated guanine are possible.

Table 1. Summary of Parameters Added to AMBER Force Field[a]

param	ideal value	force const
Pt−S01/S02 bond length	2.27 Å	360 kcal/(mol·Å^2)
Pt−S01/S02−CT	104°	62 kcal/(mol·rad^2)
Pt−S01/S02−LP	114°	150 kcal/(mol·rad^2)
CT−S01/S02−LP	114°	150 kcal/(mol·rad^2)
S01/S02−Pt−cis ligand	90°	42 kcal/(mol·rad^2)
S01/S02−Pt−trans ligand	180°	42 kcal/(mol·rad^2)

[a] See refs 18 and 19 for parameters for guanine and chloride ligands, respectively.

Likewise, we would expect that bulky amine ligands would result in interligand clashes with coordinated methionine residues. However, we hypothesize that such bulky amine ligands would cause different degrees of steric clashes with guanine compared to methionine because of the planarity of the former. Such differences could affect the relative stabilities of guanine versus methionine adducts. Furthermore, steric interactions could disfavor certain coordination stoichiometries and/or change the atoms involved in coordination.

The AMBER force field is commonly utilized in molecular mechanics calculations of DNA and protein residues.[17] This force field has been modified previously to include parameters for platinum bound to the guanine *N*7 atom[18] and to include chloride ligands.[19] We have implemented further modifications of the force field to include parameters for platinum bound to methionine. These modifications have permitted us to compare the structures of platinum diamine complexes with guanine and methionine. We have utilized these calculations to predict the relative stabilities of selected guanine and methionine complexes. We have also utilized NMR spectroscopy to test experimentally our predictions from the calculations.

Experimental Section

Commercial reagent grade chemicals were used without further purification. $Pt(en)Cl_2$ and $Pt(Me_4en)Cl_2$ were synthesized on the basis of a previous modification[19] of the method of Romeo et al.[20]

Molecular Mechanics Calculations. All molecular mechanics calculations were performed using HyperChem 7 (Hypercube, Inc.) on a Dell Optiplex GX260 computer running Windows XP. The AMBER89 force field was modified to include parameters from previous studies involving platinum guanine[18] and platinum chloride[19] complexes as well as those parameters listed in Table 1 and discussed below. The distance-dependent dielectric constant (4*r) was utilized, and the 1−4 nonbond parameters were scaled by 0.5 as is typical for AMBER.[18]

We found that the atomic charges did not greatly affect the relative energies of our complexes. We estimated the effect of platinum binding on the charges of methionine analogously to the method utilized for guanine.[18] Briefly, the methionine atoms were categorized as primary (bound to Pt), secondary (bound to primary), or tertiary (bound to secondary), and additional charge (+0.459)

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was distributed to atoms in a 10:3:1 ratio. Amine ligand charges (unplatinated) were estimated by extended Hückel calculations on an amine ligand; these unplatinated charges were modified in a manner similar to that for the methionine except that the additional charge was +0.1865 for each coordination position.

Molecular dynamics were typically run at a simulated temperature of 300 K for 250 ps; structures containing a chelated (via *S* and *N*) methionine were instead run at 1800 K for 250 ps. Structures were saved every 1 ps, and these structures were subjected to 1000 cycles of steepest decents and 10 000 cycles of Fletcher−Reeves conjugate gradient minimizations or until the gradient was <0.01 kcal/(mol·Å).

Reactions of Pt(en)Cl_2 and Pt(Me_4en)Cl_2 with 5′-GMP, Met, and/or *N*-AcMet. In a typical experiment, 5 mg of the PtA_2Cl_2 compound was reacted with $AgNO_3$ in a 2:1 $AgNO_3$:Pt molar ratio in 1 mL of D_2O. After filtration to remove the AgCl precipitate, 5′-GMP, Met, or *N*-AcMet was added to the sample and the pH was adjusted to ∼4 (all pH values uncorrected). The reactions were monitored by NMR spectroscopy.

NMR Spectroscopy. All spectra were recorded in D_2O on a JEOL 270 MHz instrument. ^{1}H NMR were referenced to the residual HOD signal (4.8 ppm). Samples for ^{195}Pt NMR (^{1}H-decoupled) were made as described above except that ∼20 mg of the platinum complexes was utilized. ^{195}Pt NMR spectra were recorded using a 1000 ppm sweep width, 2048 points, and a 0.2 s relaxation time; the spectra were referenced relative to K_2PtCl_6 (external reference, $\delta = 0$ ppm).

Results

Parameters for Molecular Modeling of Pt Methionine. We defined new potential types within AMBER for the sulfur atoms of methionine bound to platinum. We adopted a convention similar to that utilized for platinum−DNA modeling;[18] namely, S01 and S02 were defined as the sulfur atoms of coordinated methionine that are trans to N31 and N32, respectively.

Examination of several X-ray crystal structures[21−31] containing platinum−sulfur bonds have bond lengths ranging from 2.23 to 2.34 Å. Juranić et al. previously used a value of 2.30 Å for modeling [Pt(trpy)GS]$^+$.[32] We decided to use a slightly shorter value of 2.27 Å for platinum bound to methionine because structures containing a thioether[22−27] typically had bond lengths in the 2.26−2.28 Å range.

Juranić et al. utilized a force constant of 300 kcal/(mol·Å^2) for the Pt−S bond in their modeling study.[32] Yao et al. utilized force constants of 366 kcal/(mol·Å^2) for the platinum−amine bonds as well as the platinum−*N*7 bond of guanine,[18] and they noted that the use of a smaller force constant gave similar results. We decided to use 366 kcal/(mol·Å^2) for our Pt−S force constant, as this is consistent with the value used for the Pt−N force constant and is similar to that used by Juranić et al.[32] Likewise, we utilized similar right-angle parameters for cis and coaxial parameters for trans ligand bonds about the platinum that were utilized for previous modifications to AMBER.[18,19]

Formation of the Pt−S bond creates new angles. We initially used ideal angles and force constants for the Pt−S−C and Pt−S−LP (LP = lone pair) on the basis of the parameters for C−S−C and C−S−LP angles within the force field. However, when platinum coordinates to methionine, only one lone pair is left on the sulfur atom; the lack of two sets of lone pair parameters caused some angles to be incorrectly modeled. Specifically, the Pt−S−C angles were ∼5−8° larger than reported in crystal structures and the C−S−C angles were ∼15° larger than in crystal structures.[23,24,27] We found that values of 104° for the Pt−S−C angle and 114° for the Pt−S−LP and C−S−LP angles in platinum methionine structures gave more realistic values for the angles.

No parameters for torsional strain were added to the force field, since examination of the X-ray structures showed no obvious range values for the torsions. We expect that the torsions will be influenced primarily by interligand van der Waals interactions. The van der Waals radius and ϵ value of platinum used previously[18] were also utilized here. Our parameters are summarized in Table 1.

Testing Force Field Parameters. Our parameters were tested by constructing models of a number of complexes that had X-ray structures available.[23,24,26,27] Overall, we observed good agreement between the bond lengths and angles of our calculated structures and the X-ray structures. We constructed a model of [Pt($CH_3SCH_2CH_2SCH_3$)(9-EtG)$_2$]$^{2+}$ and performed a geometry optimization of the structure utilizing the AMBER force field modified to include our platinum methionine parameters as well as the previous platinum guanine parameters.[18] This structure was compared to the X-ray structure of [Pt($MeSCH_2CH_2SMe$)(5′-GMP)$_2$]·6H_2O that was previously reported.[23] The rms deviation of the heavy atoms of the dithioether ligand was only 0.05 Å, indicating excellent agreement between the calculated and X-ray structures for this portion of the molecule. The guanine bases were less canted in the calculated structure than in the X-ray structure; however, molecular packing of the unit cell may be responsible for the canting.[23]

We also constructed structures of $PtCl_2$(methionine-*N*,*S*) with both *R* and *S* chiralities at the sulfur atom for comparison to an X-ray structure[24] and modeling calculations utilizing the MM3 force field.[33] Our lowest energy structures

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Table 2. Calculated Minimum Energies of $PtA_2(9\text{-EtG})_2$, $PtA_2(Met)_2$, and $PtA_2(9\text{-EtG})(Met)$ Complexes with All Energies in kcal/mol

ligands	cisplatin energy	Me_4en energy	diff
$(9\text{-EtG})_2$	−17.66	−5.40	12.26
$(Met)_2$	−10.59	16.06	26.65
(9-EtG)(Met)	−12.35	1.90	14.25

for the *R* and *S* chiralities were −3.95 and −4.20 kcal/mol, respectively. For the *R* chirality, both the S−CH_3 and the carboxyl group were in equatorial positions; for the *S* chirality, the carboxyl group was equatorial and the S−CH_3 group was axial. Calculations that utilized MM3[33] found that the chair conformers with equatorial carboxyl groups were favored, consistent with our study. The X-ray structures[24] consisted of slightly distorted chair conformers with equatorial carboxyl groups and axial S−CH_3 groups. However, the bond lengths and angles of the X-ray structures were in reasonable agreement with our calculated structures.

Modeling of Platinum Guanine and Platinum Methionine Complexes. By utilizing the modifications made previously to the AMBER force field,[18] we were able to model $[cis\text{-}Pt(NH_3)_2(9\text{-EtG})_2]^{2+}$ and $[Pt(Me_4en)(9\text{-EtG})_2]^{2+}$ complexes. We constructed both head-to-head (HH) and head-to tail (HT) rotamers of the guanine ligands. The lowest energy conformers in each case involved an HT orientation of the 9-EtG ligands (Table 2). The lowest energies were −17.65 and −5.40 kcal/mol for $[cis\text{-}Pt(NH_3)_2(9\text{-EtG})_2]^{2+}$ and $[Pt(Me_4en)(9\text{-EtG})_2]^{2+}$, respectively. These energies cannot be compared directly, as each structure has different numbers and types of atoms. However, the energies do provide a reference point in that replacing the nonbulky ammine ligands with the Me_4en ligand led to an energy increase of ∼12.3 kcal/mol. The 9-EtG ligands in the Me_4en complex were less canted than in the diammine complex to avoid steric clashes with the Me_4en ligand.

Once the force field was modified to include platinum methionine parameters, we then constructed computer models of $[cis\text{-}Pt(NH_3)_2(Met)_2]^{2+}$ and $[Pt(Me_4en)(Met)_2]^{2+}$ complexes in which the Met ligands were bound to the platinum via the sulfur atoms. Coordination of the sulfur to platinum results in a chiral center; thus, we constructed separate structures with chiralities of *R*,*R*, *R*,*S*, and *S*,*S* at the sulfur atoms.

Rotation of the Met ligand around the Pt−S bond leads to different rotamers. For the *R*,*R* and *S*,*S* chiralities, one HH and two HT rotamers are possible, utilizing a designation similar to that of *cis*-PtA_2G_2 systems. Two HH and two HT rotamers are possible for the *R*,*S* chirality. We therefore performed calculations on our complexes for all rotamers.

The minimum energy structures of $[cis\text{-}Pt(NH_3)_2(Met)_2]^{2+}$ and $[Pt(Me_4en)(Met)_2]^{2+}$ both had the *R*,*R* chirality at the sulfur atoms and were HH; the energies were −10.59 and 16.06 kcal/mol, respectively (Table 2). Thus, replacement of the nonbulky ammine ligand with Me_4en leads to a calculated energy increase of >26 kcal/mol for the bis(methionine) complexes, significantly greater than the ∼12.3 kcal/mol calculated for an analogous change in bulk for the bis(guanine) complexes. Thus, we examined the $[Pt(Me_4en)(Met)_2]^{2+}$ structure to determine the sources of the dramatic energy increase. Severe steric crowding was apparent in the $[Pt(Me_4en)(Met)_2]^{2+}$ complex, and correspondingly there was an ∼8 kcal/mol increase in van der Waals energy compared to the $[cis\text{-}Pt(NH_3)(Met)_2]^{2+}$ complex. Angle strain was also significant; for example, one Pt−S bond was not coplanar with the rest of the platinum coordination plane, with cis and trans S−Pt−N angles of 100.9 and 168.1°, respectively.

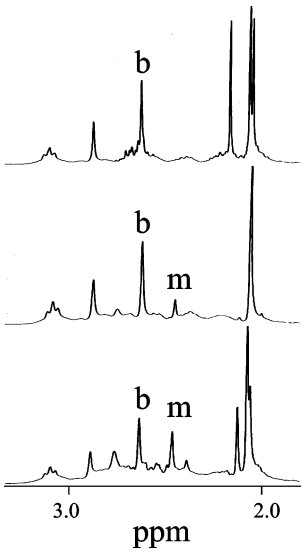

Figure 2. Partial NMR spectrum of the reaction of $Pt(en)(D_2O)_2$ with *N*-acetylmethionine. The letters "b" and "m" represent the δ-CH_3 signal of the bis and mono products, respectively. Bottom: ∼2:1 *N*-AcMet:Pt ratio, $t = 1$ h. Middle: ∼2:1 *N*-AcMet:Pt ratio, $t = 1$ day. Top: ∼4:1 *N*-AcMet:Pt ratio, $t = 1$ day.

Because our calculations suggested that the $[Pt(Me_4en)(Met)_2]^{2+}$ complex would be highly strained, we examined other possible $Pt(Me_4en)$ complexes with methionine. We constructed a $[Pt(Me_4en)(Met\text{-}S,N)]^{+}$ chelate and subjected the structure to molecular mechanics and dynamics. The lowest energy structures had the six-membered ring that resulted from Met chelation in a chair conformation. The δ-CH_3 groups were in the axial positions regardless of the sulfur chirality; the axial position of the δ-CH_3 groups reduced interligand steric clashes. The minimum energies for the complexes with the *R* and *S* chiralities of the sulfur atoms were 16.79 and 15.92 kcal/mol, respectively. The corresponding lowest energy structures of $[cis\text{-}Pt(NH_3)_2(Met\text{-}S,N)]^{+}$ were calculated to be 3.34 and 4.69 kcal/mol. The differences in energy upon the change from the diammine to the Me_4en ligand (∼13.5 and 11.3 kcal/mol, respectively) are similar to those observed for $[cis\text{-}PtA_2(9\text{-EtG})_2]^{2+}$.

Reaction of $Pt(en)(D_2O)_2$ with *N*-AcMet. We utilized $[Pt(en)(D_2O)_2]^{2+}$ as our nonbulky platinum complex in experimental studies because the chelated diamine ligand is less likely to be displaced as a result of the trans effect of a coordinated sulfur.[3] *N*-Acetylmethionine was added to a $[Pt(en)(D_2O)_2]^{2+}$ sample in a ∼2:1 ratio, and an NMR spectrum was collected at a pH of ∼4 (Figure 2); *N*-acetylmethionine was initially chosen instead of methionine because it has been utilized in similar studies[14] with $Pt(en)Cl_2$ and because the methyl signal from the acetyl group provides another NMR

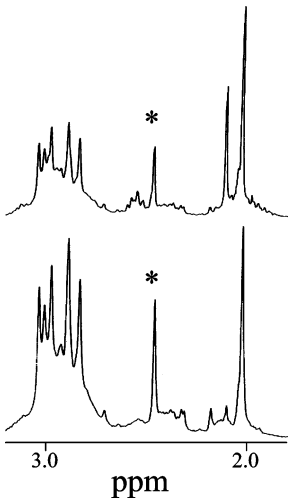

Figure 3. Partial NMR spectrum of the reaction of *N*-acetylmethionine with $Pt(Me_4en)(D_2O)_2$ at 1:1 (bottom) and >2:1 (top) *N*-AcMet:Pt ratios after 1 day. The starred signal indicates the δ-CH_3 signal of the product.

signal that is readily observable. On the basis of the comparison to previous results,[14] we assigned the new sets of resonances that appeared to $[Pt(en)(N\text{-AcMet-}S)(D_2O)]^{2+}$ and $[Pt(en)(N\text{-AcMet-}S)_2]^{2+}$. In the early stages of the reaction, the set of resonances corresponding to the former were dominant. When the reaction was complete, the signals of the latter were dominant and only a trace amount of the former remained. Additional *N*-acetylmethionine was added to the sample, and the signals of $[Pt(en)(N\text{-AcMet-}S)(D_2O)]^{2+}$ disappeared completely.

Reaction of $Pt(Me_4en)(D_2O)_2$ with *N*-AcMet. When *N*-AcMet was added to the $[Pt(Me_4en)(D_2O)_2]^{2+}$ sample at a 1:1 ratio and a pH of ~4, a new set of NMR resonances was observed (Figure 3). A signal at 2.42 ppm was assigned to the δ-CH_3 (S−CH_3) signal; such a downfield chemical shift is similar to that observed for the $[Pt(en)(N\text{-AcMet-}S)Cl]^+$ complex[14] and other sulfur-coordinated methionine residues.[16]

When additional *N*-AcMet was added to a sample (at pH 4) to create a 2:1 *N*-AcMet:Pt ratio, no new signals were observed even after several weeks. Thus, only one product is formed even in the presence of 2 equiv of *N*-AcMet. The assignment of this product to a mono(methionine) product is based on several observations. First, this product (and only this product) is observed at Pt:Met ratios of 1:2, 1:1, and 2:1; in contrast, for $[Pt(en)(D_2O)_2]^{2+}$ and *N*-AcMet, the mono product dominates at low *N*-AcMet:Pt ratios whereas $[Pt(en)(N\text{-AcMet-}S)_2]^{2+}$ dominates at high *N*-AcMet:Pt ratios. Second, the stoichiometry of reaction and the integration of the NMR signals indicate a 1:1 ratio of platinum to methionine. Third, the chemical shift of the δ-CH_3 signal of *N*-AcMet is 2.42 ppm, which is close to that observed to the δ-CH_3 signal of the mono- but not the bis-*N*-AcMet complex formed from $Pt(en)Cl_2$.

Our experimental results support our MMD studies, which suggested that a bis(methionine) complex would not form. Because the chemical shift of the δ-CH_3 signal indicates sulfur coordination, three types of complexes are possible: $[Pt(Me_4en)(N\text{-AcMet-}S)(D_2O)]^{2+}$; $[Pt(Me_4en)(N\text{-AcMet-}S,O)]^{2+}$; $[Pt(Me_4en)(N\text{-AcMet-}S,N)]^+$. Examination of the $[Pt(Me_4en)(\text{Met-}S,N)]^+$ structures generated by MMD calculations suggested that formation of a *S*,*N* chelate would not be expected when an acetyl group is on the nitrogen due to steric crowding between the acetyl group and the Me_4en ligand. Furthermore, a ^{195}Pt NMR signal was observed for the complex at −2760 ppm, only slightly upfield of the ca. −2600 to −2700 ppm range observed previously for a PtN_2SO coordination sphere.[15] Our signal is significantly downfield of the ca. −3000 to −3300 ppm range observed for PtN_3S or ca. −3600 to −3800 ppm for PtN_2S_2 coordination spheres.[15,16]

A doublet of doublets centered at ~5.65 ppm was observed and assigned to α-H; such a shift has been observed for α-H signals of *S*,*O* chelates with methionine and *S*-methylcysteine where a carboxyl oxygen atom is involved in the chelate formation.[15,34] Thus, our product is assigned to be a $[Pt(Me_4en)(N\text{-AcMet-}S,O)]^{2+}$ chelate. Previous *S*,*O* chelates have shown two sets of NMR resonances due to slow inversion of the sulfur chirality.[34] Only one product was observed for our chelate. This observation suggests that either one diastereomer dominates or that the two diastereomers could not be resolved.

When the pH of a sample of $[Pt(Me_4en)(N\text{-AcMet-}S,O)]^{2+}$ was lowered to ~2, we observed new resonances appearing at 2.95 and 3.6 ppm; these resonances cooresponded to the CH_3 and CH_2 resonances, respectively, of uncoordinated Me_4-en. The rate of appearance of these signals was greater at lower pH values, indicating the dissociation of the Me_4en ligand is acid-catalyzed. Dissociation of ammine or ethylenediamine ligands upon coordination of a sulfur-bound ligand in the trans site has been observed previously.[3,16,35]

Reaction of $Pt(Me_4en)(D_2O)_2$ with 5′-GMP. We added 2 equiv of 5′-GMP to a sample of $[Pt(Me_4en)(D_2O)_2]^{2+}$. New resonances in the NMR spectrum began to appear within 10 min; eventually two new sets of resonances dominated. The chemical shifts of the *H*8 resonances were 8.44 and 8.45 ppm at pH 3; when the pH was raised to 7, the *H*8 resonances were at 8.34 and 8.41 ppm, respectively. These signals were assigned to $[Pt(Me_4en)(5'\text{-GMP-}N7)_2]$ (charge depends on pH) on the basis of the 2:1 GMP:Pt stoichiometry and comparison of the integration of the *H*8 and Me_4en signals; *N*7 coordination is indicated by the downfield shift of the *H*8 signals.[5−8] The presence of two sets of resonances of unequal intensities indicates slow exchange between two HT rotamers. Such an observation is in agreement with previous studies of $[Pt(Me_4en)(guanosine)_2]^{2+}$.[4]

Formation of $Pt(Me_4en)$(*N*-AcMet)(5′-GMP). We then wanted to see if 5′-GMP could displace *N*-AcMet. We expected that the Pt−O bond would be easier to displace; thus, $[Pt(Me_4en)(N\text{-AcMet-}S)(5'\text{-GMP})]^+$ was a possible product. We constructed computer models of $[cis\text{-}Pt(NH_3)_2(\text{Met-}S)(9\text{-EtG})]^{2+}$ and $[Pt(Me_4en)(\text{Met-}S)(9\text{-EtG})]^{2+}$, and we observed all possible rotamers as well as both chiralities of

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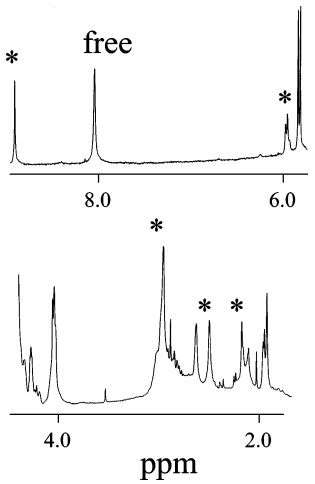

Figure 4. NMR spectrum of Pt(Me$_4$en)(*N*-AcMet-*S*,*O*) after the addition of 2 equiv of 5′-GMP. Spectra were collected after 18 h at 37 °C. The H8 signal due to free 5′-GMP is labeled "free", and peaks that were not observed in spectra of Pt(Me$_4$en)(*N*-AcMet-*S*,*O*) or Pt(Me$_4$en)(5′-GMP)$_2$ are marked with an asterisk.

the Met ligands. The lowest energies of these mixed-ligand conformers were −12.36 and 1.90 kcal/mol, respectively, a difference of ∼14.3 kcal/mol (Table 2). This difference is reasonably close to the energy difference (12.3 kcal/mol) calculated for the bis(guanine) complexes, suggesting the mixed-ligand complex would not have severe steric clashes.

We then added 2 equiv of 5′-GMP to a sample of [Pt(Me$_4$en)(*N*-AcMet-*S*,*O*)]$^{2+}$. After 18 h at 37 °C, new signals were observed in the NMR spectrum (Figure 4). A new resonance in the *H*8 region of the spectrum was observed at 8.9 ppm that was approximately equal in intensity to the *H*8 signal of unreacted 5′-GMP; such a resonance (at 8.9 ppm) does not correspond to [Pt(Me$_4$en)(5′-GMP)$_2$], suggesting it is due to [Pt(Me$_4$en)(*N*-AcMet)(5′-GMP)]$^+$. The significant downfield shift of the *H*8 atom indicates *N*7 coordination of the 5′-GMP. Likewise, the δ-CH$_3$ signal of [Pt(Me$_4$en)(*N*-AcMet-*S*,*O*)]$^{2+}$ at 2.42 ppm was no longer visible, and two resonances at 2.16 and 2.2 ppm had grown. The presence of a PtN$_3$S coordination geometry was supported by a ^{195}Pt NMR shift at −3082 ppm. The ^{1}H NMR signals did not disappear even after several days, and no [Pt(Me$_4$en)(5′-GMP)$_2$] signals appeared, indicating complete displacement of *N*-AcMet did not occur.

Reaction of [Pt(Me$_4$en)(D$_2$O)$_2$]$^{2+}$ with Met. When 1 equiv of methionine was added to [Pt(Me$_4$en)(D$_2$O)$_2$]$^{2+}$, two new signals in the NMR spectrum at 2.58 and 2.61 ppm were assigned to δ-CH$_3$ resonances (Figure 5). Addition of another 1 equiv of methionine did not result in the appearance of any new signals. The δ-CH$_3$ resonances are downfield of the value of ∼2.4 ppm that was observed when *N*-AcMet was added to [Pt(Me$_4$en)(D$_2$O)$_2$]$^{2+}$. Chemical shifts of ∼2.5−2.6 have been commonly observed for methionine residues that are chelated to platinum via the S and N atoms.[15,35] Also, a small broad cluster of signals at ∼3.55 ppm integrate to a 1:3 ratio relative to the δ-CH$_3$ resonances and are assigned to the α-H resonances; this upfield shift from ∼3.8 ppm for the α-H resonance in free methionine is comparable to the ∼0.3 ppm shift observed for this signal in other *S*,*N*

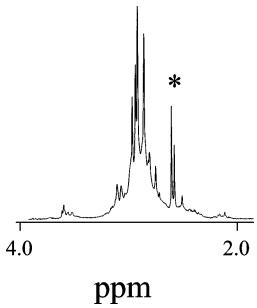

Figure 5. Partial NMR spectrum of Pt(Me$_4$en)(D$_2$O)$_2$ after the addition of 1 equiv of methionine. The product δ-CH$_3$ signals are represented by an asterisk.

chelates.[34,35] Furthermore, similar chelates have resulted in multiple isomers due to slow isomerization of the sulfur chirality and/or adoption of cis or trans isomers about the amide bond.[14,35] Thus, these new resonances are assigned to a *S*,*N* chelate; the presence of two sets of signals may be due to slow isomerization of the sulfur chirality, although a slow exchange between conformations of the six-membered chelate ring cannot be ruled out. Support for the assignment of *S*,*N* chelates comes from the ^{195}Pt NMR spectrum, which shows two signals at −3160 and −3230 ppm; these signals are in the expected range for a PtN$_3$S coordination geometry.

We prepared a new sample of [Pt(Me$_4$en)(Met-*S*,*N*)]$^+$ and added 2 equiv of 5′-GMP to the sample; no new resonances appeared even after 7 days at 37 °C and a pH of 4.3. This observation suggests that the *S*,*N* chelate is more stable than the *S*,*O* chelate that formed with *N*-acetylmethionine.

Discussion

Molecular mechanics calculations of platinum guanine complexes have been utilized extensively.[18,19,36] Such calculations have led to insights regarding the favored conformations of guanine bases and the amine ligands of the platinum. In contrast, relatively few studies have focus on the modeling of platinum coordinated to amino acids. Because platinum has a high affinity for coordination to sulfur, cysteine and methionine are expected to be primary sites of protein adduction by platinum. We chose to focus on platinum methionine complexes for this study.

A previous study utilized the MM3 force field to model PtCl$_2$(L-methionine-*N*,*S*).[33] We chose instead to modify the AMBER force field that is commonly used for modeling DNA and proteins so that our parameters may be utilized for modeling platinum adducts with larger peptides and/or proteins. Previous modifications to this force field have allowed the inclusion of platinum guanine[18] and platinum chloride[19] compounds. Our current modifications further extend this force field to include platinum methionine complexes. When all of these modifications are incorporated, complexes containing nearly any combination of amine, guanine, chloride, or methionine ligands can be studied.

Calculations utilizing the bulky Me$_4$en ligand led to the prediction that [Pt(Me$_4$en)(Met-*S*)$_2$]$^{2+}$ complexes would be energetically unfavorable due to steric clashes. This predic-

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tion was supported by our experimental results. Both Met and *N*-AcMet react with a 1:1 stoichiometry with Pt(Me_4en). In contrast, guanosine[4] and 5′-GMP can react in a 2:1 guanine:Pt(Me_4en) ratio. Thus, the Me_4en ligand disfavors the 2:1 complex for methionine but not guanine ligands.

Nonbulky platinum compounds such as *cis*-Pt(NH_3)$_2$$Cl_2$ and Pt(en)Cl_2 often react to form *S,N* chelates with methionine residues containing either amine or amide functionalities at the *N*-terminus.[14−16] In contrast, our NMR results indicated significant differences between the Met and *N*-AcMet complexes that formed from reaction with [Pt(Me_4en)(D_2O)$_2$]$^{2+}$. Specifically, two sets of NMR resonances appeared for the Met complexes and the chemical shifts of the δ-CH_3 resonances were relatively downfield; an upfield shift of the α-H signal was also noted. Only one new set of NMR resonances was observed for the *N*-AcMet reaction, and a significant downfield shift of the α-H signal was noted. These observations suggested that Met formed a *S,N* chelate whereas *N*-AcMet formed a *S,O* chelate. Such assignments were supported by the ^{195}Pt NMR shifts. Thus, the presence of the acetyl group changes the nature of the chelate formed. Molecular mechanics calculations suggest that a *S,N* chelate would be relatively free of strain for methionine. We did not develop force field parameters for modeling platinum bound to an amide nitrogen because of the lack of suitable X-ray structures of platinum coordinated to an amide; however, by examining the [Pt(Me_4en)(Met-*S,N*)]$^+$ structures, we conclude that steric crowding between the acetyl group of *N*-AcMet and the Me_4en ligand would be unavoidable.

Previous studies found that the *N*-methyl groups of a *N,N*′-dimethyl-2,4-diaminopentane ligand had axial positions in platinum complexes[7,19] to avoid interligand clashes. Likewise, our calculations of [Pt(Me_4en)(Met-*S,N*)]$^+$ suggest that the δ-CH_3 group has an axial orientation to avoid interligand clashes. For $PtCl_2$(Met-*S,N*), the δ-CH_3 group was axial for the *S* chirality and equatorial for the *R*; the former was calculated to be very slightly lower in energy.

For methionine-containing peptides and proteins, chelation of the sulfur and nitrogen of the methionine residue is common.[15,35,37] In contrast, [Pt(Me_4en)(D_2O)$_2$]$^{2+}$ cannot form such a chelate unless the methionine residue has a free amine group. *S,O* chelation has been observed kinetically for a peptide with a C-terminal methionine residue, but this chelate was short-lived relative to other products.[35] It is therefore very interesting that a *S,O* chelate is the dominant product observed for the reaction of [Pt(Me_4en)(D_2O)$_2$]$^{2+}$ and *N*-AcMet. Platinum and/or palladium complexes can catalyze cleavage of peptide bonds on the C-terminal side of methionine.[11,12] Indirect evidence suggests that such cleavage occurs through attack of an external water molecule on a carbonyl carbon whose oxygen is coordinated to the metal center;[38] such cleavages are often done at acidic pH to reduce the amount of competing *S,N* chelate.[11] We expect that Pt(Me_4en) complexes would be unable to form such a *S,N* chelate in a protein, and it is thus possible that these complexes may be suitable for chemical cleavage of peptide bonds.

Displacement of the coordinated nitrogen by 5′-GMP was observed for [Pt(en)(*N*-AcMet-*S,N*)]$^+$.[14] In contrast, displacement of the nitrogen of [Pt(Me_4en)(Met-*S,N*)]$^+$ did not occur when 5′-GMP was added, perhaps due to the fact that an amine nitrogen rather than an amide nitrogen is coordinated in the [Pt(Me_4en)(Met-*S,N*)]$^+$ chelate. For [Pt(Me_4en)(*N*-AcMet-*S,O*)]$^{2+}$, 5′-GMP can displace the oxygen of the chelate to form a [Pt(Me_4en)(*N*-AcMet-*S*)(5′-GMP)]$^+$ complex. Displacement of the sulfur to form the bis-5′-GMP complex was not observed within 24 h. By comparison, Sadler et al. reported relatively rapid formation of [Pt(en)(*N*-AcMet-*S*)(5′-GMP)]$^+$ but only slow formation of a small amount of [Pt(en)(5′-GMP)$_2$] when 5′-GMP was added to [Pt(en)(*N*-AcMet-*S,N*)]$^+$ or [Pt(en)(*N*-AcMet-*S*)$_2$]$^{2+}$.[14] Formation of a *cis*-Pt(NH_3)$_2$(5′-GMP)$_2$ complex via displacement of methionine-containing peptides has been observed for cisplatin and carboplatin.[39]

A previous study[40] found that *cis*-PtA_2Cl_2 complexes varied in their reactivities with the Met1 residue of ubiquitin. Specifically, complexes with nonbulky amine ligands reacted preferentially with this methionine residue, whereas those complexes with bulky ligands did not. Since this methionine residue has a free amine, our results suggest that a *S,N* chelate could be formed even for bulky platinum complexes. Thus, the lack of reaction with this methionine residue by bulky complexes may be due to steric clashes with nearby residues and/or other kinetic factors.

In summary, we have utilized a modified AMBER force field to study the effects of amine ligand bulk on the relative stabilities of platinum diamine complexes with guanine and methionine. Experimentally, we have found that the type of platinum diamine complexes that form with methionine differ as a result of amine ligand bulk. The Me_4en ligand limits the stoichiometry to 1:1, and *S,N* or *S,O* chelates are the dominant products in the absence and presence of an acetyl group, respectively.

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Supporting Information Available: Tables of minimum calculated energies for all possible chiralities and conformers of PtA_2(9-EtG)$_2$, PtA_2(Met)$_2$, and PtA_2(9-EtG)(Met). This material is available free of charge via the Internet at http://pubs.acs.org.

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