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Molecular Mechanics Analysis of Platinum Compounds Chelated to Methionine Residues

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Thesis for the Western Kentucky University Honors Program

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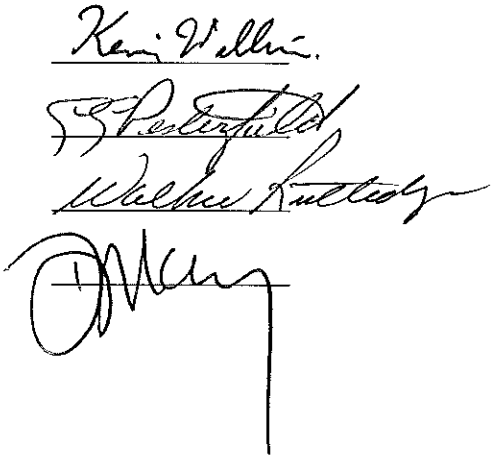

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Abstract

Recently, interest in certain platinum compounds has increased significantly due to the discovery that they have either anticancer properties, proteolytic properties, or both. In order to find platinum compounds with similar properties that work better or can be synthesized less expensively, one can use computerized molecular mechanics can be used. In this study a modified AMBER force field was used to find the most stable conformations of platinum (II) N, N-diethylethylenediamine (Et_2en) compounds chelated to various amino acid residue combinations. Using comparisons with other platinum compounds, and a bis-guanine chelate as the standard for all compounds, this research found that conformations were preferred that positioned most of the bulk of the chelate away from the diethyl group of the Et_2en. It was also found that sulfur-oxygen chelates of methionine are more preferred over bis-methionine structures. When additional amino acid residues were connected to the methionine in the sulfur-oxygen chelates, they did not play a substantial role in increasing the energy of the overall structure. The additional amino acids did, in some cases, lower the energy of the structure due to their ability to form strong intramolecular electrostatic interactions. It should be noted that work from another study confirms the data acquired in this one that sulfur-oxygen chelates are preferred, as well as the particular type.

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Introduction

Heavy metals have a bad reputation for being detrimental to the health of humans. However, there are certain platinum-containing compounds that have been found to have beneficial biochemical and medicinal properties. For example, cisplatin and its analogs have been found to have anticancer capabilities and are very useful in treating testicular, ovarian, lung, urethral, skin, gastrointestinal tract, and in the head and neck (Lippert, 37-54). Other platinum compounds aid in protein hydrolysis, which is important for a variety laboratory applications (Milovic, 4037). The mechanisms for these activities involve the platinum atom of these compounds chelating to DNA and proteins. For anticancer activity, the platinum attaches to guanine nucleotides and distorts the shape of the DNA molecule. Evidence for this damage causing cell death comes from studies involving prophage-infected bacteria (Reedijk, 2501). When protein cleavage occurs, the platinum will chelate to a methionine residue in the protein and catalyze hydrolysis.

With chemicals there is constant work being done to improve what exists, or to find new substances that will do the function of current substances better or cheaper. To aid in the search these new compounds, researchers can utilize molecular mechanics modeling calculations. This modeling helps to pinpoint potentially useful compounds and can often conserve time and resources that would be spent doing laboratory

that would find the same information. These molecular modeling calculations can also act as a confirmation for data that are found in the laboratory.

The underlying purpose in performing the molecular modeling work is to find the geometry of a molecular structure that confers the minimum energy for the structure. This is done by using a collection of programmed parameters, called a forcefield, which represents bond lengths, bond angles, torsional strain, and electrostatic interactions (Höltje and Folkers, 13). Such parameters calculate the energy of a conformation. The modeling program can use the forcefield parameters in order to determine the most stable conformation. This process ends when slight alterations in the conformation do not lower the energy further. Since it is possible for the different starting geometries to produce different minimum energies, a procedure needs to be included that produces many different starting geometries in order for one to increase the chance of finding the absolute minimum energy for the structure according to the parameters (Comba and Hambley, 62-63).

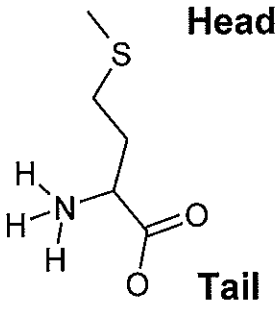
In this study, three main types of complexes were examined. All involved an atom of platinum (II) chelated to N, N – diethylethylenediamine (Et_2en). The complex types differed in their other ligands, which were bis-methionine, bis-guanine, and a single methionine amino acid chelated to platinum via the sulfur atom and a carboxylic oxygen. There were also different conformations for the structures that could be made for each complex type that needed to be examined. For example, there are four possible configurations for a bis-methionine complex, based on the chirality of the sulfur atoms in the methionines. Additionally, each configuration has four possible conformational forms based on the directions of the heads and tails of the methionines (the head being

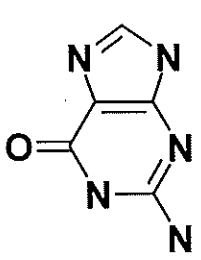
the methyl group bound to the sulfur and the tail being the rest of the amino acid). The bis-guanine complex comes in four conformations based on the directions of the heads and tails of the nucleotide bases (the head for guanine is the C8 atom, and the rest of the nitrogenous base is the tail). Finally, there are four configurations of the sulfur-oxygen chelate of the single methionine amino acid which differ by the positioning of the sulfur coordinated to the platinum and the chirality of the sulfur. Two tripeptides containing methionine were also examined. These tripeptides were chelated to platinum in the same fashion as the single methionine amino acid. The difference between the two was that one had two glycines surrounding a methionine, while the other had two isoleucines surrounding a methionine. Glycine was chosen for the tripeptide examinations because it is the smallest amino acid. The choice of isoleucine was based on its being an amino acid with large steric hindrance, and being non-polar in its R-group. The nomenclature regarding the examined structures followed these basic rules:

1.) The ligand cis to the nitrogen without the ethyl groups was reported first in describing the molecules.

2.) The H/T (head/tail) designation indicated which portion of the molecule was below the platinum plane when viewed from the methionine or guanine side of the complex with the diethyl group to the right.

3.) For the sulfur-oxygen chelates, the cis and trans designation showed where the sulfur atom was in relation to the nitrogen on the carrier ligand to which the ethyl groups were bound.





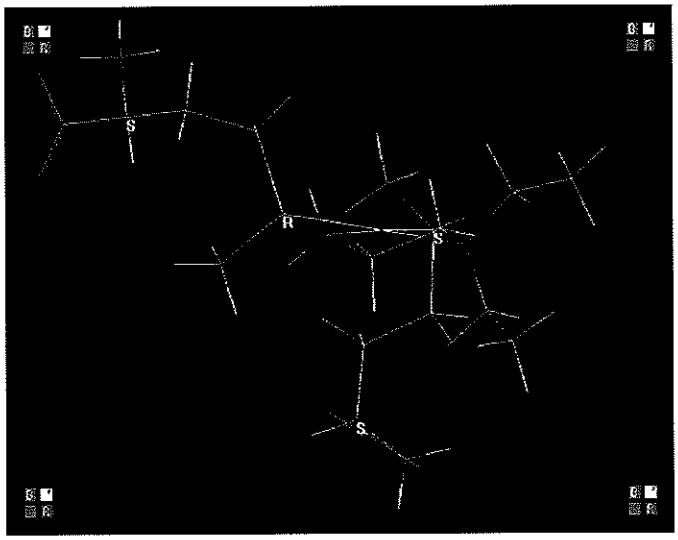
A.) **Methionine**

Guanine

B.)

C.)

D.)



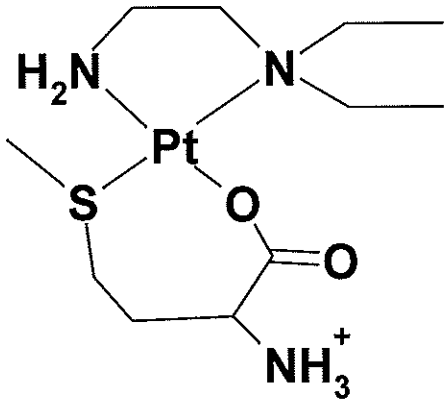


Figure I:

Parts A and B show methionine and guanine with the head and tail labeled. Part C shows the nomenclature methods in work for a bis-methionine structure. The structure shown is an R,S-H,T bis-methionine. Part D is an example of a cis sulfur-oxygen chelate of methionine. The tripeptides will have the additional amino acids attached where the free carboxylic oxygen is present and in place of all but one of the amino terminal hydrogens.

Most of the work was performed with Et_2en as the carrier ligand. Data used with regard to cisplatin and N, N, N', N'-tetramethylethylenediamine (Me_4en) were taken from Williams et al. *Inorganic Chemistry*, **2004**, *43*, 1190-1196. The data involved bis-methionine and bis-guanine. The information regarding sulfur-oxygen chelates came

from the current study. Cisplatin and Me_4en are symmetrical; therefore, there are no trans or cis designations in their nomenclature for sulfur-oxygen chelates. Only the chirality of the sulfur atom in the methionine amino acid was used to designate the form of the structure.

From the outset, this research anticipated that the steric hindrance and asymmetry of Et_2en would prevent the formation of certain configurations or conformations of the guanine and methionine complexes. This might lead to configurations of bis-methionine, bis-guanine, and sulfur-oxygen chelates that could be unstable or have high energies due to steric hindrance from Et_2en. Another examination of preferred binding involved the study of how the surrounding amino acid residues in a polypeptide chain would affect the affinity of methionine to form sulfur-oxygen chelates with platinum. It was expected that the additional amino acids would affect the ability of platinum and methionine to bind.

Experimental Procedure

Variations to the standard parameters for the AMBER forcefield were used in this study. Many were preexisting parameters found in publications by Williams and Yao, and involved parameters for the binding of guanine and methionine to a platinum atom. The new parameters developed for this study included the bond length of a carboxylic oxygen atom bound to a platinum atom (2.01 Å) and the size of the angle between the carbon, oxygen, and platinum atoms (115.89°) that results from the binding. The values for the parameters came about through taking the averages of x-ray crystallography data for platinum compounds with carboxyl oxygen chelates. These values were found in publications by Ali, Hall, Kortes, Mukhopadhyay, and Watabe.

All modeling was performed on the Hyperchem 7 modeling program by Hypercube, Incorporated. Finding the minimum energies for all of the structures being examined required that the structures first be constructed in the computer. Then a geometric optimization was performed on the structure until it reached its minimum energy. The next step was to perform a molecular dynamics run on the structures. This was a simulated heating of the structures for 250 picoseconds at 300 Kelvin. Snapshots of the structures were taken every picosecond, and each snapshot underwent geometric

optimization. The minimum energy for a structure was the lowest energy reported after all of the snapshots were optimized.

The above procedure provided the minimum energy for a particular structure. The minimum energy was actually the sum of different energies that corresponded to the bonds, molecular strain, and forces of attraction and repulsion. While the Hyperchem program calculated these energies, they were reported as the minimum energy in the Hyperchem window. The mechanism which allowed for these individual energies to appear involved making a computational log, which is a text file showing all of the computations that the program performed for the structure. In order to create a log, one instructs the program to start a log and then tells it to calculate the minimum energy for the structure on the screen (Hypercube, 130).

Data and Analysis

The energies that were obtained for different carrier ligands could not be directly compared to each other because of the difference in the number of atoms that each contained. Instead, the structures with a particular carrier ligand were compared to the minimum energy obtained from the bis-guanine structures. Then the differences between the minimum energy of the bis-guanine and that of the other structures were calculated. Differences in energy could be compared since the calculation of the differences factors out the dissimilarities with the carrier ligands. The calculated differences appear on the table below along with the minimum energies, strain energies, and attractive energies of the examined structures.

There was a special calculation that was performed (see Table I.). It involved those values labeled as S-O Core for the tripeptides. These were measurements acquired through the creation of a log for the minimum energy of the structures with only the platinum atom, carrier ligand, and the atoms of the methionine residue selected for calculations to be performed on. This calculation analyzed what changes in the energy of the structure occurred from the addition of the other two amino acids when compared to the single methionine sulfur-oxygen chelate.

The minimum energy calculations for the cisplatin and Me_4en as the carrier ligands chelated with bis-methionine and bis-guanine was taken from older work that was performed by the author's research group. Because of the inaccessibility of the said research, the strain and attractive energies of these structures were not available. The strain energies of the examined structures were the sums of the bond, angle, dihedral, and van der Waals energies of the structures. The energies from hydrogen bonding and electrostatic interactions were summed together to provide the attractive energies of the structures. All of the energies reported were in kilocalories per mole.

Table I

Structure	Total Energy	Strain Energy	Attractive Energy	Total Energy-BisGuanine
Bis-Guanine (Minimum Energy)				
Et_2en	-4.90576			
Cisplatin	-17.6564			
Me_4en	-5.39912			
Et_2en Bis-Guanine				
H,H	-4.905761	13.72865	-18.634375	-1E-06
H,T	-3.639201	15.00601	-18.645248	1.266559
T,H	-4.669875	13.84946	-18.51930365	0.235885
T,T	-1.893838	15.56907	-17.46290217	3.011922
Et_2en Bis-Methionine				
R,R Chirality				
H,H	8.278421	18.77635	-10.497899	13.184181
H,T	18.917576	28.27511	-9.357565	23.823336
T,H	19.353125	31.44342	-12.090308	24.258885
T,T	19.17992	32.14184	-12.962016	24.08568
R,S Chirality				
H,H	31.812052	36.98613	-5.174082	36.717812
H,T	5.410873	18.22305	-12.812216	10.316633
T,H	16.276291	30.80144	-14.525197	21.182051
T,T	19.368664	32.66403	-13.295353	24.274424
S,R Chirality				
H,H	22.063604	33.51622	-11.452613	26.969364
H,T	16.163342	30.75521	-14.591834	21.069102
T,H	4.366611	20.05116	-15.684575	9.272371
T,T	17.92271	32.69487	-14.7721	22.82847
S,S Chirality				
H,H	16.384121	29.58513	-13.201022	21.289881
H,T	19.129026	32.9802	-13.851237	24.034786
T,H	11.945357	29.89558	-17.950317	16.851117
T,T	4.628458	10.63383	-13.005355	9.534218
Sulfur Oxygen Chelate				
Single Methionine				
Cisplatin R chiral	7.367361	12.195188	-4.827839	25.023761
Cisplatin S chiral	4.736063	10.461038	-5.724976	22.392463
Et_2en R cis	21.331648	27.232440	-5.900747	26.237408
Et_2en R trans	19.215317	21.788810	-2.573479	24.121077
Et_2en S cis	20.222635	25.956320	-5.733644	25.128395
Et_2en S trans	16.025984	22.088240	5.592172	20.931744
Me_4en R chiral	17.918043	24.155804	-6.237756	23.317163
Me_4en S chiral	19.168215	26.395030	-7.226811	24.567335
Glycine-Methionine-Glycine				
Cisplatin R chiral	-1.990154	16.578551	-18.56865	15.666246
Cisplatin S chiral	0.294913	14.747975	-14.45305	17.951313
Et_2en R cis	17.739756	33.865490	-16.12574	22.645516
Et_2en R trans	9.532526	24.081100	-14.54864	14.438286
Et_2en S cis	12.229028	30.201060	-17.97199	17.134788
Et_2en S trans	15.236161	28.711670	-13.475572	20.141921
Me_4en R chiral	19.401886	32.079480	-12.67752	24.801006
Me_4en S chiral	14.220384	28.446860	-14.226509	19.619504

Isoleucine-Methionine-Isoleucine				
Cisplatin R chiral	-6.058862	17.515253	-23.574132	11.597538
Cisplatin S chiral	-4.045775	23.646276	-27.69206	13.610625
Et_2en R cis	-0.252210	29.901100	-30.153325	4.653550
Et_2en R trans	-0.235844	32.234310	-32.470165	4.669916
Et_2en S cis	9.034516	39.007510	-29.973006	13.940276
Et_2en S trans	9.246382	36.058270	-26.811869	14.152142
Me_4en R chiral	9.029792	36.552800	-27.522995	14.428912
Me_4en S chiral	15.950630	38.813390	-22.862667	21.349750
Glycine-Methionine-Glycine S-O Core				
Cisplatin R chiral	7.052561	15.203494	-8.150919	24.708961
Cisplatin S chiral	9.497588	12.726702	-3.229111	27.153988
Et_2en R cis	27.050737	32.549800	-5.499083	31.956497
Et_2en R trans	18.752359	22.737540	-3.985217	23.658119
Et_2en S cis	20.866873	28.394520	-7.527673	25.772633
Et_2en S trans	24.864580	28.068030	-3.203444	29.770340
Me_4en R chiral	29.039738	31.074280	-2.034543	34.438858
Me_4en S chiral	25.110210	28.015330	-2.905113	30.509330
Isoleucine-Methionine-Isoleucine S-O Core				
Cisplatin R chiral	-9.594158	9.460494	-19.054664	8.062242
Cisplatin S chiral	-4.817144	16.390291	-21.207417	12.839256
Et_2en R cis	10.480736	23.081140	-12.600440	15.386496
Et_2en R trans	10.382479	23.711850	-13.329319	15.288239
Et_2en S cis	19.981762	32.313000	-12.331248	24.887522
Et_2en S trans	8.916098	28.324560	-19.408464	13.821858
Me_4en R chiral	7.539129	28.076090	-20.536973	12.938249
Me_4en S chiral	12.267915	30.327690	-18.059765	17.667035

Conclusions and Discussions

From what has been observed, steric hindrance, electrostatic interactions, and structural strain play a large role in providing the energy of a structure. In the bis-methionine structures, the conformations that were favored were those that could reduce steric hindrance by pointing the bulk of the methionine residue away from the ethylenediamine ring and the ethyl groups on that ring. For the sulfur-oxygen chelates, the strain in the platinum-methionine ring ended up being a large influence in the energy. Also, the position of the sulfur atom in the sulfur-oxygen chelates provided a portion of the strain energy, especially when the sulfur atom is in the cis configuration. An examination to see how much the extra amino acids in the tripeptides contributed to the strain energy of the structure showed that while they contributed to a small increase in the strain energy of the structure, they did not play a role in determining the relationship of the strain energies for related structures.

Electrostatic interactions, along with hydrogen bonds, made the structures more stable and therefore decreased the overall energy of the structures. There were conformations with total energies that were the lowest for their carrier ligand but had higher strain energies than other conformations. Most of the electrostatic interactions came from the carboxyl and amino terminals of the amino acids. This was even more

prevalent in the tripeptides due to the two additional amino acids being able to bend into geometries that caused the terminals of the peptides and internal amide groups to have greater access to each other. There is the possibility that if the modeled tripeptide chelates were to be dissolved in a polar solvent that electrostatic interactions with the solvent would be preferred to the observed intramolecular electrostatic interactions.

When examining the differences between the minimum energies for the bis-guanine structures of the different carrier ligands, and the energies of the bis-methionine and sulfur-oxygen chelates, this research found that Et_2en was more favorable than the Me4en when it came to the bis-methionine, yet neither of those two appeared to be better than cisplatin. As for the sulfur-oxygen chelates, the differences between the minimum bis-guanine energies and the energies of the sulfur-oxygen chelates for all carrier ligands were relatively close to each other, suggesting the sulfur-oxygen chelates are relatively strain-free. If the minimum energies for the sulfur-oxygen chelates for each carrier ligand type were used, then it would appear that Et_2en would be preferred, followed by cisplatin, and then Me_4en.

An initial goal was to be able to examine sulfur- nitrogen chelates. However, difficulty in developing an adequate parameter for the platinum bound to an amide nitrogen surfaced. This was due to literature concerning x-ray crystallography with amide nitrogen atoms bound to platinum being inconsistent with the nature of the bond between the nitrogen and carbon of the amide functional group. Some publications showed this bond to be a single bond, while others showed it to be a double bond. Also, there were differences in the bond lengths between the single bond and the double bond.

Developing a working parameter is something that should be done sometime in the near future.

In a related project, performed by a coworker in the laboratory, involving the kinetics of platinum with an Et_2en carrier ligand, binding to N-acetylmethionine formed only sulfur-oxygen chelates experimentally. The difference between N-acetylmethionine and regular methionine is that N-acetylmethionine is a form of methionine with an acetyl group bound to the amino terminus of the methionine to simulate a peptide bond. Examination, via NMR, showed that even with an excess of N-acetylmethionine, no bis-methionine structure would form. Because sulfur is a more polarizable atom, it reacts with platinum faster than oxygen. Thus the position occupied by the oxsygen atom is the one not selected by the sulfur atom. This observation of inaccessibility, coupled with the location of peaks on the NMR, showed that the sulfur-oxygen chelate would be in the trans conformation, and that steric hindrance from the ethyl groups of the Et_2en blocked sulfur from binding in the cis position. Also, in the NMR data, there were two peaks in several places showing a slight variation in the structures sampled in the NMR that corresponded to two chiralities of the sample being present.

The above finding lends support to the data acquired in the molecular mechanics study. The preference of the trans conformation would be thermodynamically more stable according to the data from molecular modeling of the same structure. Also, the variation of the structures could be due to variations in the chirality of the sulfur atom in the methionine. The collected data showed that the differences in the energies between the two conformations might be close enough to allow for one to not be entirely excluded when the sulfur-oxygen chelate is formed.

There is future work that can be carried on from this project. First would be to see the boundaries in the background conditions at which the sulfur-oxygen chelates can form. Wet chemistry would need to be performed on the tripeptides that were modeled so that the kinetics and behavior of the sulfur-oxygen chelate for the tripeptide could be found. Furthermore, different combinations of positioning and composition of methionine containing peptide chains can be modeled.

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