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Reactions With Platinum (ll) Complexes And Selenium-Containing Amino Acids

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REACTIONS WITH PLATINUM (ll) COMPLEXES AND SELENIUM-CONTAINING AMINO ACIDS

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By

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ABSTRACT

We have reacted $[Pt(Me_4en)(D_2O)_2]^{2+}$ [Me_4En=N,N,N'N'-tetramethylethylenediamine] with Selenomethionine (SeMet), Methionine (Met), and Methylselenocysteine (MeSeCys). When MeSeCys was reacted with $[Pt(Me_4en)(D_2O)_2]^{2+}$, we observed both stereoisomers of Se,N chelates, as well as $[Pt(Me_4en)(MeSeCys)Cl]^+$ from ^1H NMR Spectroscopy; the latter formed due to the presence of Cl^- in the solution. Both isomers of the chelate seemed to form proportionally to one another, not favoring a specific stereoisomer. Eventually the $[Pt(Me_4en)(MeSeCys)Cl]^+$ products became Se,N chelates. We incubated SeMet with NaCl for 30 minutes and then mixed with $[Pt(Me_4en)(D_2O)_2]^{2+}$; we saw equal amounts of the $[Pt(Me_4en)(SeMet)Cl]^+$ isomers along with a specific stereoisomer of the Se,N chelate forming first (R chirality), then approximately two hours later the (S) chirality formed. Previously, Met and $[Pt(Me_4En(D_2O)_2]^{2+}$ have been studied by prior students and there were no chiralities favored in the reaction. There were S,N chelates formed, but no specific isomer favored over another. We obtained equal amounts of SeMet, Met and $[Pt(Me_4en)(D_2O)_2]^{2+}$ and mixed the solutions together to see which amino acid would platinate first, either SeMet or Met. The NMR spectra we observed showed that SeMet attached first to $[Pt(Me_4en)(D_2O)_2]^{2+}$, with the (R) chelate forming first, then the (S)

chelate. Met was slow to react, and we saw both chelates form at approximately equal rates.

Keywords: Cisplatin, Selenomethionine, Selenocysteine, Bulky Platinum (ll) Compounds

Dedicated to my friends, family and my professors.

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TABLE OF CONTENTS

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

Cancer affects all races, ethnicities, genders and all locations of the world. There are many types of cancers, some are more predominant in particular genders or races than any others, some are slow growing while some spread very rapidly. According to the National Cancer Institute of the Surveillance Epidemiology and End Results Statistics Fact Sheet it is estimated that 11,957,599 men and women in the United States have had a history of cancer by January 1, 2008.[1] There are a few different options to try to treat and cure the cancer, but there is still ongoing research to come up with more effective yet less toxic treatments. One popular area of anticancer medication research involves the use of platinum (II) compounds. Currently there are a few platinum anticancer medications FDA approved for the treatment of cancers; cisplatin, carboplatin, and oxaliplatin. Unfortunately, like many medications, side effects, resistance and toxicity become a problem with these medications.

Figure 1:

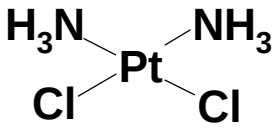


Figure 1 contains the structure of Cisplatin (cis-diamminedichloroplatinum (II)).

Cisplatin (cis-diamminedichloroplatinum (II)) was the first platinum anticancer medication approved for the treatment of cancer by the FDA in 1978.[2] The anticancer activity was discovered by accident in the 1960's by Barnett Rosenberg when he was trying to monitor cell growth and division of the bacteria *E. coli*.[2] The cell division was inhibited while the cell growth was not bothered.[2] This was later tested in sarcomas in rats and it was found that the platinum (II) complex was very effective in reducing the mass of the tumors.[2] This finding led to other conformational testing in various tumor cell lines and eventually led to the FDA approval of the medication for testicular and ovarian cancers.[2]

The anticancer activity comes from the platinum drug binding to DNA at the N7 position of the guanine residue on the double helix, forming a crosslink. The formed crosslink forces the double helix to bend in a distorted manner, which leads to apoptosis. In addition to monoadducts, there are three different types of possible crosslinks that can be formed giving rise to the anticancer activity: intrastrand crosslink, interstrand crosslink and a DNA-protein crosslink.[3] Typically a monoadduct is initially formed, but then the complex goes on to form one of the other three crosslinks.[3] High mobility group proteins are also thought contribute to the anticancer activity.[3] These proteins bind to the platinum-DNA crosslink not allowing the cell to participate in repair mechanisms.[3]

A theory in chemistry called the Hard Soft Acid Base Theory; also known as the donor-acceptor theory, helps explain why the platinum (II) complexes react with particular amino acids and molecules. The theory was developed in the 1960's by Ralph Pearson.[4] The idea helps explain many reactions in chemistry; a base is defined as an electron-donor and an acid is defined as an electron-acceptor.[4] A covalent bond joins the

two together and forms an adduct, complex or a coordination complex.[4] When a particular acid can accommodate one base (ligand) it is often referred to as a monodentate ligand. Where as if there is more than one donatable nonbonding electron pairs it is often referred to as either polydentate, bi-, tri-, tetra-, hexa- dentate, or chelating ligand.[4] The HSAB Theory helps in forming an idea as to what adduct will form after a reaction because of classifying atoms as either hard/soft acids or bases. Typically hard acids are atoms that have a relatively high charge ($\geq 3^+$), small size and low electronegativity (.7-1.6).[4] Particularly atoms located in the s and f blocks, and higher charged ions on the left of the d block have low electronegativity.[4] Hard bases usually have a high electronegativity and are small in size. There are only 2 atoms within the electronegativity range of 3.4-4, oxygen and fluorine.[4] So, hard bases are typically ones with either oxygen or fluorine as the donor atom. Soft acids are typically classified as large size (1^+, 2^+), low charge and intermediate to high electronegativity (1.9-2.5).[4] Specific examples include Hg^{2+}, Cu^+, and Ag^+.[4] Soft bases include those that have intermediate to high electronegativity (2.1-3.0) and are large in size leading to polarizability.[4] Particular examples of soft bases include S^{2-}, Se^-, I^-, and Br^-.[4]

Platinum is considered a "soft acid" under the Hard/Soft Acid/Base Theory, which means the platinum is more likely to react with "soft bases." The atoms become "softer" going down the columns, thus, sulfur is softer than oxygen and selenium is softer then sulfur. These particular atoms are important because two amino acids contain a sulfur atom, methionine and cysteine, as well as a nitrogen and oxygen atom. Selenium is of importance because two other amino acids contain a selenium atom, selenomethionine and selenocysteine. Selenomethionine is produced unintentionally in

the body while selenocysteine is made deliberately; however, it is still unknown the purpose and function of selenomethionine. Selenocysteine, however, is currently being called the 21st amino acid, and is known for its antioxidant properties. Since selenium is softer then sulfur, it would be predicted that the selenium containing amino acids would be kinetically favored in a platinum (II) complex rather than the sulfur containing amino acids. There are proteins in the body that contain either one or more of these selenium containing amino acids, which would also be thought that the platinum compound could react with these proteins before it even reaches the DNA[5.] Typically it is thought that the major mechanism of cell death is the binding of the platinum complex to DNA, it is also possible that part of the mechanism could be contributed by the protein reacting with the platinum compound as well[5].

Figure 2:

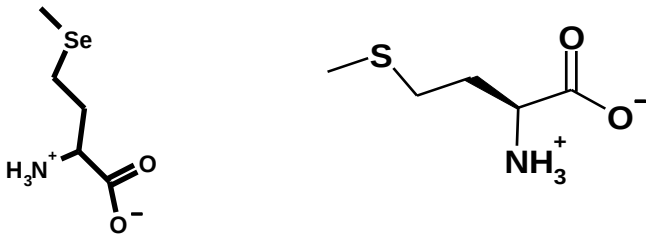


Figure 2 shows the selenomethionine and methionine molecules.

Once cisplatin enters the blood stream it remains with its initial ligands as shown in figure 1. The Cl^- concentration is so high in the blood stream that the chlorides remain attached to the platinum, leaving the compound at a neutral charge.[5] Since it's now neutrally charged, the complex can enter the cytosol of the cell via the cell membrane. Once this occurs, the chloride ligands are displaced by water molecules, now leaving the

complex positively charged giving it the option to react with the DNA or proteins.[5] If cisplatin reacts with DNA it either leads to cell repair or cell death, while if it reacts with proteins this can trigger toxicity and/or resistance.[5]

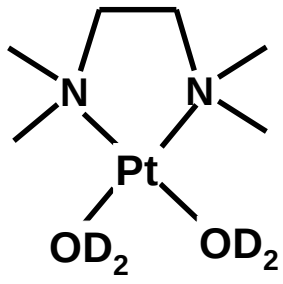
As with any drug in the medical field there are many side effects with the use of cisplatin, including but not limited to, nephrotoxicity, hematological toxicity, ototoxicity, neuropathy and seizures. Also, as with many chemotherapy agents, all cells in the body are affected by the antitumor drug. Cisplatin affects the cancerous cell as well as other normal, healthy cells in the body. With all of these many and harsh side effects on the body, researchers are trying to develop a better, less toxic anticancer medication. It is thought that toxicity is created by competitive protein binding and could possibly be controlled with a rescue agent being given in conjunction with the platinum therapy.[6] A rescue agent is considered to be a sulfur-containing ligand, that can improve side effects without affecting the antitumor activity, by either preventing or reversing the Pt-S bond in proteins.[6] But first, one must understand how the side effects are caused in the body, what biomolecules are being affected. With basic coordination chemistry S-donor ligands in proteins will rapidly produce the most stable bonds with Pt complexes. It can also react with the lone pairs of N atom in amino acid side chains. There are a few amino acids that contain an S-donor ligand, including cysteine and methionine, that are located in particular proteins.

As stated earlier, selenium is "softer" than sulfur according to the hard-soft acid-base (HSAB) theory. Since it's already known that Pt has a high affinity for sulfur atoms[6], it could potentially also have a high affinity for selenium-containing amino acids, selenomethionine and selenocysteine. When Pt binds to DNA, it typically always reacts

with 2 adjacent guanines (specifically the N(7) atom because its more nucloephilic) more predominantly than with anything else. Because of the square planar geometry of cisplatin, when it bonds to guanine, the Pt-G bond rotates fast and spins freely. To slow the rotation of the bond, bulk was added to the cisplatin molecule creating a new platinum complex $[Pt(Me_4en)(D_2O)_2]^{2+}$ (Me_4en=tetramethylethylene diamine). When the $[Pt(Me_4en)(D_2O)_2]^{2+}$ is reacted with guanine, it slows down the rotation spin so that one can characterize the products.[3] Adding the bulk to the platinum compound has delivered many results when reacted with guanine, as well as amino acids. For example, when $[Pt(Me_4en)(D_2O)_2]^{2+}$ is reacted with excess methionines, a bis-product is formed.[5] When reacting selenomethionine with the same platinum complex, the results are not the same. So, with this it is important to study how these platinum compounds with bulky ligands react with these amino acids. Using bulky platinum compounds with amino acids has given insight to the kinetics and thermodynamics of the reaction.

Figure 3

a)



b)

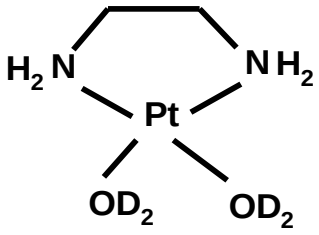


Figure 3 shows the structure of a) $[Pt(Me_4en)(D_2O)_2]^{2+}$ N,N,N',N',-tetramethylethylenediamine platinum (ll) diaqua, and b) $[Pt(en)(D_2O)_2]^{2+}$ ethylenediamine platinum (ll) diaqua

CHAPTER 2

MATERIALS AND METHODS

Synthesis of Pt compounds: All platinum compounds used were synthesized by other students in the Dr. Williams' lab based on methods of Williams et al.[6] For $[Pt(Me_4en)(D_2O)_2]^{2+}$, all solutions were made as a 10 mM solution, using 4.4 mg of the platinum compound and 1 mL of deuterium oxide. $[Pt(en)(D_2O)_2]$ solutions were made using 4.1 mg of the platinum compound in 1mL of deuterium oxide.

Preparation of solutions: All amino acid containing compounds in solutions were all made to be around 5 to 10 mM, and around a pH of 4 to 5. For the reaction involving SeMeCys and $Pt(Me_4en)$, the platinum compound was made as a 10 mM solution using 4.4 mg of platinum compound in 1 mL of deuterium oxide. SeMeCys was made as 1.4 mg of amino acid in 1 microliter of deuterium oxide giving a 10 mM solution. The final solution was made to be 300 microliters of each mixed together to give a final concentration of 5 mM.

For the reaction involving selenomethionine, sodium chloride and $[Pt(Me_4en)(D_2O)_2]^{2+}$, 0.3 mg of sodium chloride was mixed with the platinum compound and allowed to mix for 30 minutes. Selenomethionine was made using 4.1 mg of selenomethionine in 1 mL of deuterium oxide. Then using theselenomethionine at pH 4, it is mixed with the sodium chloride and platinum solution; 300 microliters were used of each solution to mix. Also, we wanted to mix the same reactants together, but add the

sodium chloride to the solution at a different time. For this reaction, 500 microliters of the selenomethionine solution and added 0.3mg of sodium chloride and allowed to mix for 30 minutes and achieve a final pH of 7.66. Then, adding 300 microliters of this SeMet-NaCl solution, to 300 microliters of the $[Pt(Me_4en)(D_2O)_2]^{2+}$ solution.

The solutions of SeCys with Pt(en) were made at different ratios, for the 2:1 ratio solutions they were made via the following reaction. 1 mg of SeCys was first mixed in 1mL of deuterium oxide and then the pH was raised to 7, then 2.5 mg of the platinum compound was added. The pH was then checked and raised back to a pH of around 7. The second 2:1 solution the pH was not raised back up to 7 after adding the platinum compound, it was left around a pH of 3. For the 1:1 solutions, 1 mg of SeCys was mixed with 1 mL of deuterium oxide and then the pH of was raised to 7 to allow for solubility. Then 1.2mg of platinum compound was raised to the SeCys solution made and the pH was then raised back up to 7. The pH of the second 1:1 solution was not adjusted back to 7, it was left at around a pH of 3.

The solutions containing SeMeCys and Pt(en) were also made at different pH's. The first solution was made by 1.1 mg SeMeCys in 1 mL of deuterium oxide and then 2.5 mg of Pt(en) were added and left at pH 3. The second solution was made using same amount SeMeCys and same amount of the platinum compound except the pH was then raised back up to 7.

CHAPTER 3

RESULTS

Reaction of MeSeCys and $[Pt(Me_4en)(D_2O)_2]^{2+}$. The addition of methyl-selenocysteine (MeSeCys) to $[Pt(Me_4en)(D_2O)_2]^{2+}$ led to a mono adduct at the Se atom; figure 5 shows the mono adduct. The MeSeCys used in the experiment contains the chloride ion in the commercial sample. When Se binds to Pt this is what leads to the two different isomers seen in the NMR spectrum. Initially, both isomers of the mono adduct are seen, $Pt(Me_4en)(MeSeCys\text{-}Se),(D_2O)^{2+}$. With the chloride in the mixture the mono adduct formed as well, $[Pt(Me_4en)(Cl)(MeSeCys)]^{+}$, with a characteristic singlet at 2.45 ppm and 2.31 ppm using ^1H NMR. Eventually, the chloride ions dissociate from the complex allowing both isomers of the *Se,N* chelate to form, $Pt(Me_4en)(MeSeCys\text{-}Se,N)^{+}$, at 2.56 ppm and 2.49 ppm. Over time the chloride adducts were forming proportionally to the MeSeCys chelates, and that both isomers of the *Se,N* chelate were forming relatively proportional to one another. With both ^1H NMR signals from the *Se,N* chelate forming at about the same rate, this is associated with no particular stereoisomer being preferred during the reaction. Eventually, both of the chloride mono adduct signals slowly diminish giving rise to more *Se,N* chelates forming and allowing free chloride to form.

Figure 4:

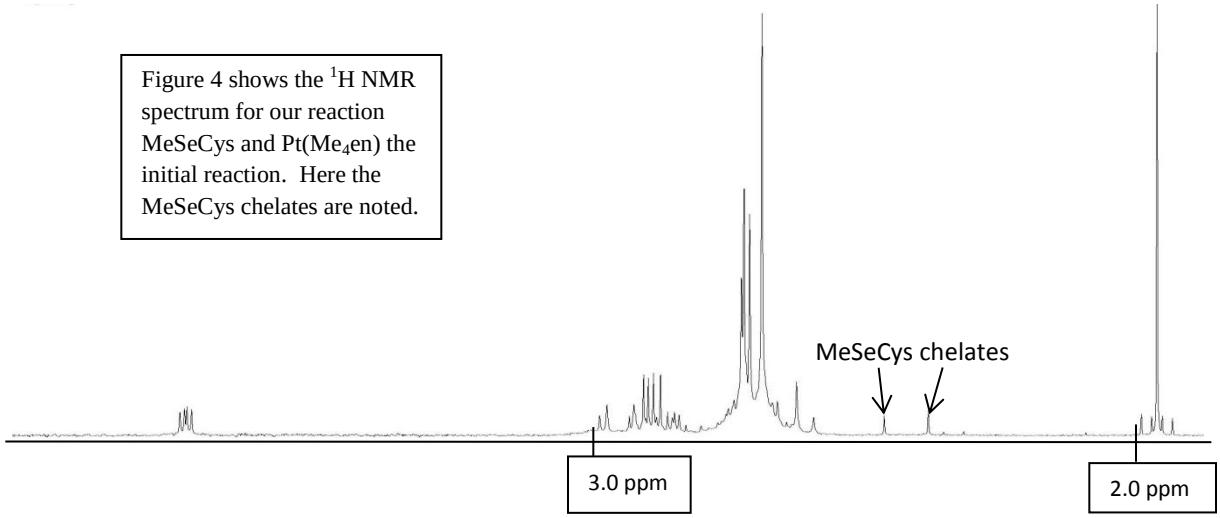


Figure 5:

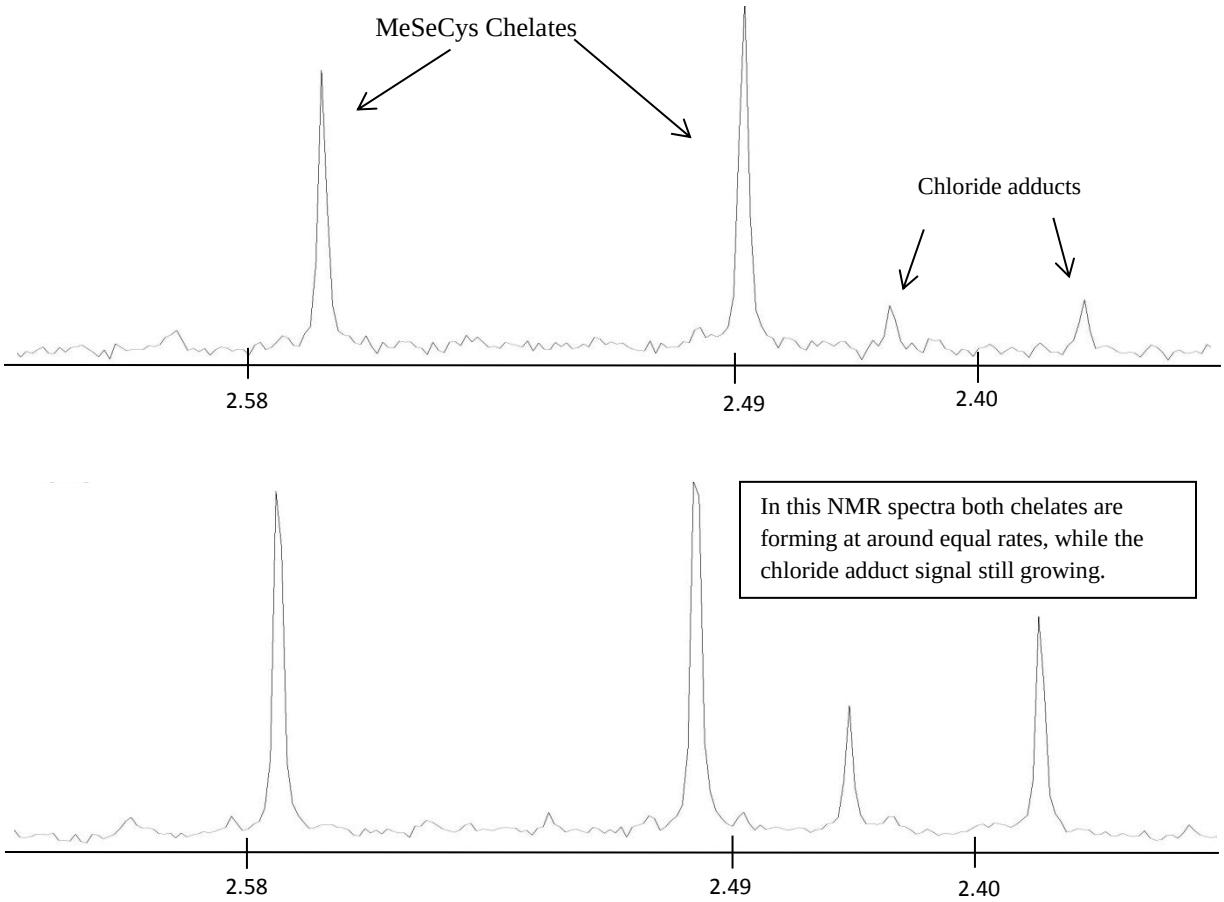


Figure 5: Overall in these spectrum from the kinetics run, the MeSeCys chelates that have formed, no isomer is preferred when forming. Also seen here the chloride adducts form, but eventually convert to all MeSeCys chelates.

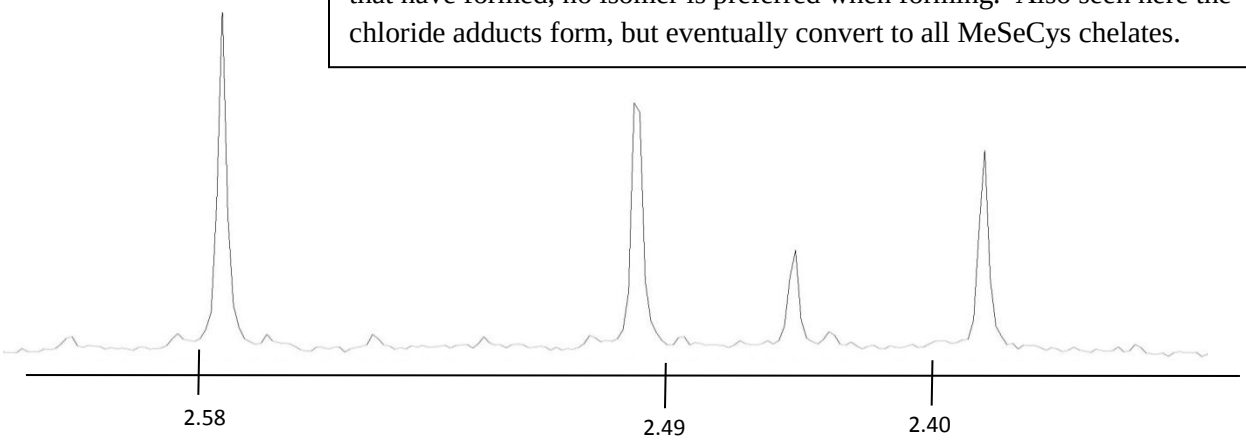


Figure 6:

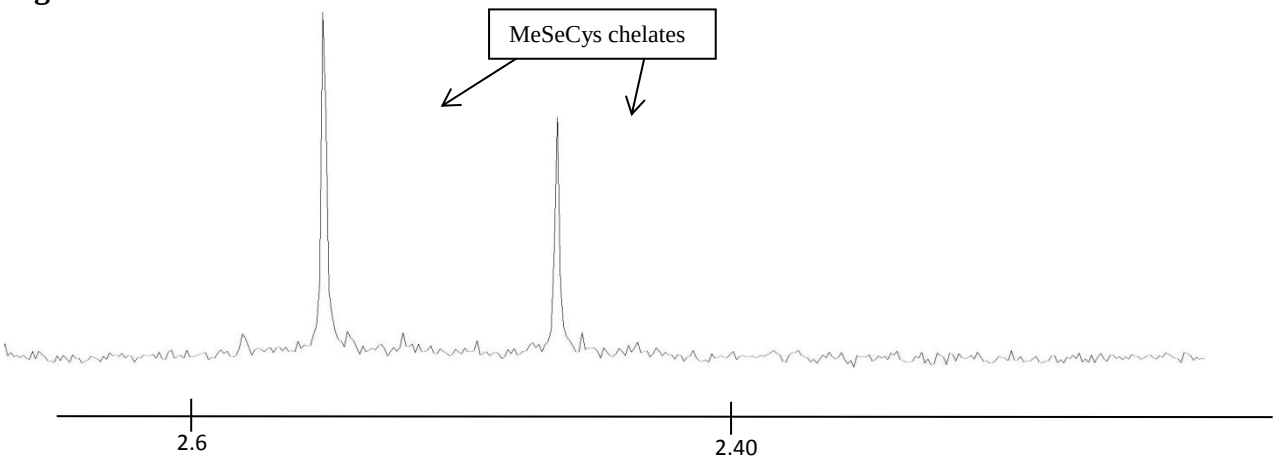


Figure 6: Here, approximately 24 hours later, the chloride adducts have converted to all MeSeCys chelates in solution.

Reaction of $[Pt(Me_4en)(D_2O)_2]^{2+}$, SeMet and NaCl. When the last experiment with MeSeCys allowed both isomers of the *Se,N* chelate to be seen, it was decided to add NaCl to $Pt(Me_4en)(D_2O)_2^{2+}$. After approximately 30 minutes, the chloride mono adduct is

forming, $[Pt(Me_4en)(Cl)(D_2O)]^+$, associated with ^1H NMR singlets at 2.84 ppm and 2.85 ppm.[6] When adding the $[Pt(Me_4en)(Cl)(D_2O)]^+$ to SeMet, two peaks are seen at 2.38 and 2.39 ppm that grew at approximately equal rates and formed $[Pt(Me_4en)(SeMet\text{-}Se)Cl]^+$.[6] After approximately 5 days the chloride ligand dissociate from the complex and allow the *Se,N* chelate to form singlets at approximately 2.58 ppm and 2.48 ppm by ^1H NMR $[Pt(Me_4en)(SeMet\text{-}Se,N)]^+$.[6]

The same experiment was completed with equal molar concentrations but changed what the incubated NaCl was mixed with. First, NaCl was reacted with SeMet for 30 minutes and then added $[Pt(Me_4en)(D_2O)_2]^{2+}$. Immediately the form mono-chloride adducts with singlets at 2.38 and 2.39 ppm, $[Pt(Me_4en)(SeMet\text{-}Se)Cl]^+$, and the same *Se,N* chelates at 2.58 and 2.48 ppm. With both isomers of the chelate, $[Pt(Me_4en)(SeMet\text{-}Se,N)]^+$ forming singlets at 2.58 and 2.48 ppm on the ^1H NMR spectrum. First, the *R* isomer shows up immediately and a little of the *S* isomer is a little slower reacting. After approximately two hours both isomers have formed equal amounts. Here it's discovered that since there are the two isomers showing with a preference for one over the other, obviously one is easier to form than the other. With the methyl group of the SeMet giving direction towards the bulky Me_4en ligand in the (*S*) position, it is less likely to form due to the bulkiness of the ligand and because of the sterics that are created when the *Se,N* chelate forms. However, when the methyl group is directed outward from the Me_4en ligand, the chelate is easier to form, thus giving a larger singlet signal on the NMR spectrum.

Figure 7:

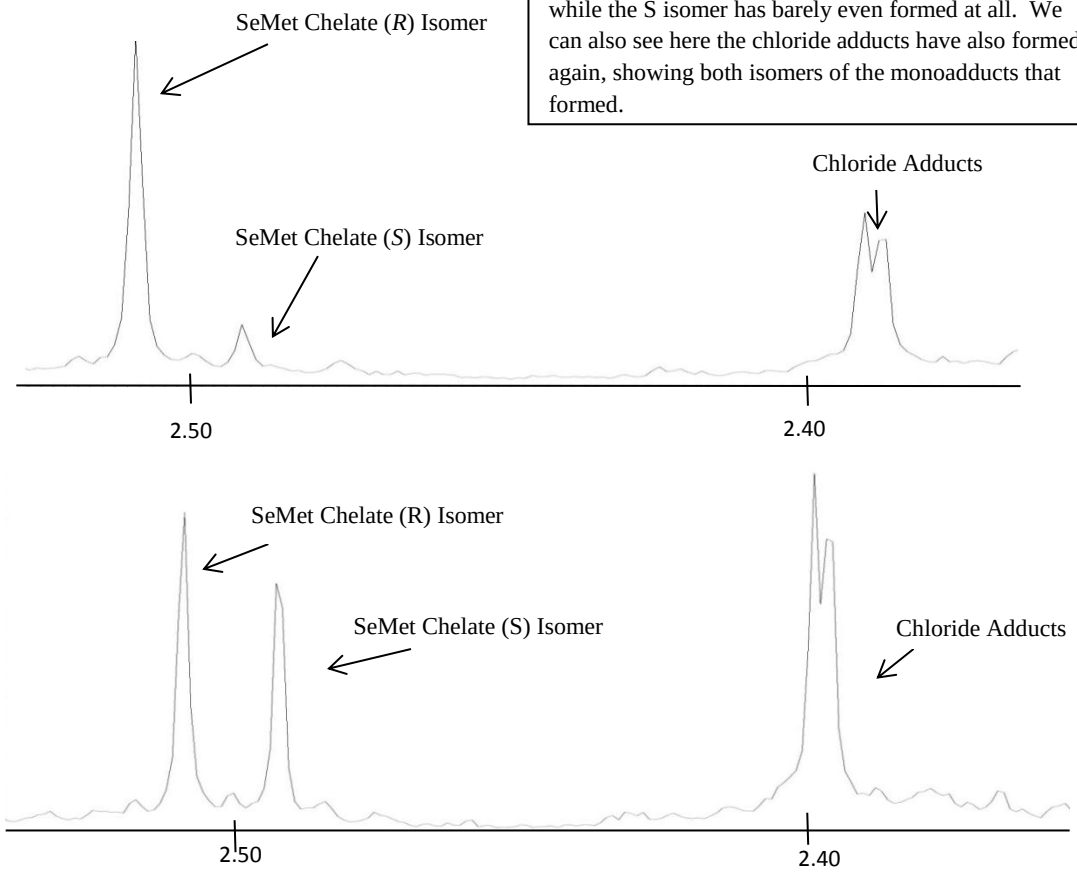


Figure 7: Here we see at the begininning of the reaction the R isomer is already predominiately formed, while the S isomer has barely even formed at all. We can also see here the chloride adducts have also formed again, showing both isomers of the monoadducts that formed.

Reaction of $[Pt(en)(NO_3)_2]^{2+}$ and SeCys: In the following reactions, SeCys was added to D_2O and then the pH was raised to 7 to allow SeCys to fully dissolve in solution[7]. After pH was adjusted and SeCys was in solution, $[Pt(en)(NO_3)_2]^{2+}$ was added. When the platinum complex was added to the SeCys the pH would immediately drop to approximately 3. Then either the solution was left at pH 3 or brought back up to around pH 7. There were four different solutions made, two pH 7 solutions and two pH 3 solutions, all with different amounts of $[Pt(en)(NO_3)_2]^{2+}$.

The first solution made was a 2:1 ratio, meaning there were two platinum complexes for every one selenocysteine complex in solution, and the pH was brought back up to 7. This would allow for one platinum atom for every one selenium atom in solution. The second solution made was also brought back up to pH 7, but this was made as a 1:1 ratio, meaning there was one platinum complex for every one selenocystine complex. This gives one platinum atom per two selenium atoms in solution. The initial color of both solutions was yellow, approximately four days later the solution had turned colorless with black precipitate in it. The pH of the 2:1 solution dropped to 4.40 after four days, while the 1:1 solution dropped to pH 5.30 after four days. The ^{1}H NMR spectrum showed that the en ligand stayed on both platinum compounds during the reaction because there were no peaks at or around 3.33 ppm, which is typically where the singlet for the en ligand would be.

Both of the pH 3 solutions were made the same way, one was a 2:1 ratio at pH 3 and the other was a 1:1 ratio at pH 3. Initially the solutions were colorless with no precipitate, while four days later both solutions had turned yellow with black precipitate in it. The color changes led to checking the pH of both solutions and in fact the pH had dropped. For the solution that was a 2:1 ratio the solution dropped to 1.90, while the 1:1 ratio solution was 2.90. Taking a look at both solutions after another four more days and the solution had turned a darker yellow-brown color with the black precipitate still in it. The pH of both solutions was checked again, while the 2:1 ratio solution had remained the same, the 1:1 ratio had actually increased to 3.50. After running both solutions in the NMR, it was noted that the en ligand had come off of the platinum compound by the

singlet at 3.33[8]. Typically the ligand dissociation requires two *Se* ligands to be coordinated to the complex.

Both solutions that were initially at pH 7 were dropped to pH 3 to see if the en ligand would dissociate from the platinum complex. Both ratios of the platinum complex were left the same, but adjusted the pH back to 3, and both solutions remained colorless even after the pH adjustment was made. Initially with the NMR spectrum there were no new peaks noted, however, a few peaks on the spectrum continuously decreased in size when it reached 48 hours after making the pH adjustment. There is a singlet at 2.33 ppm that occurs at pH 7 with both ratios of solutions; however, after dropping the pH back down to 3, the singlet eventually completely diminishes. Continuously monitoring this reaction for about a week, the en ligand never comes off of the platinum complex.

Reaction of SeMeCys and $[Pt(en)(NO_3)_2]^{2+}$: The last experiment gave the idea to try SeMeCys and the same platinum complex used above in the next reactions. For one solution the pH was left at 3 after mixing in the platinum complex, and then raised the pH of the other solution back to 7 after mixing in the platinum complex.

The first solution was made at a pH of 3, and an overnight kinetic reaction was ran for 12 hours on the ^{1}H NMR. Based upon the singlet at 3.33 ppm, this shows that the en ligand comes off of the main platinum compound. The peak is seen pretty quickly in the reaction, actually almost immediately.

The second solution was made and then raised back up to a pH of 7 and a proton kinetics run was completed. With the solution being at a higher pH than the previous

solution, the en ligand never comes off. Comparing data from the initial run and a few weeks later, the 3.33 ppm peak never shows up.

With both solutions, a significant amount of unreacted SeMeCys and Pt(en) was seen during the course of both reactions. These reactions also help reiterate that the en ligand comes off at a low pH, and doesn't at a higher pH.

Figure 8:

Figure 8: This is the initial mixing of the pH 3 sample of MeSeCys and Pt(en) for the NMR

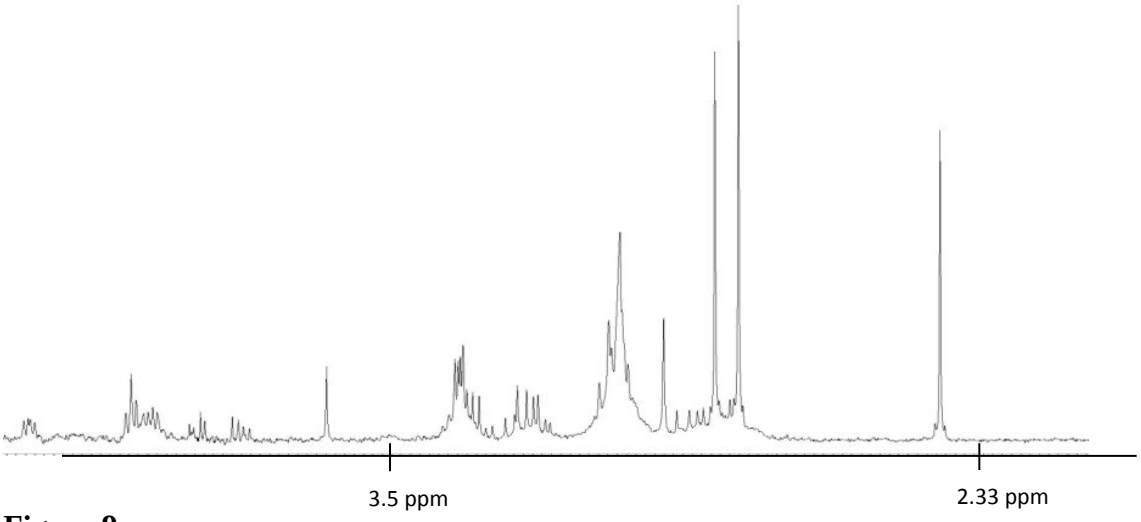
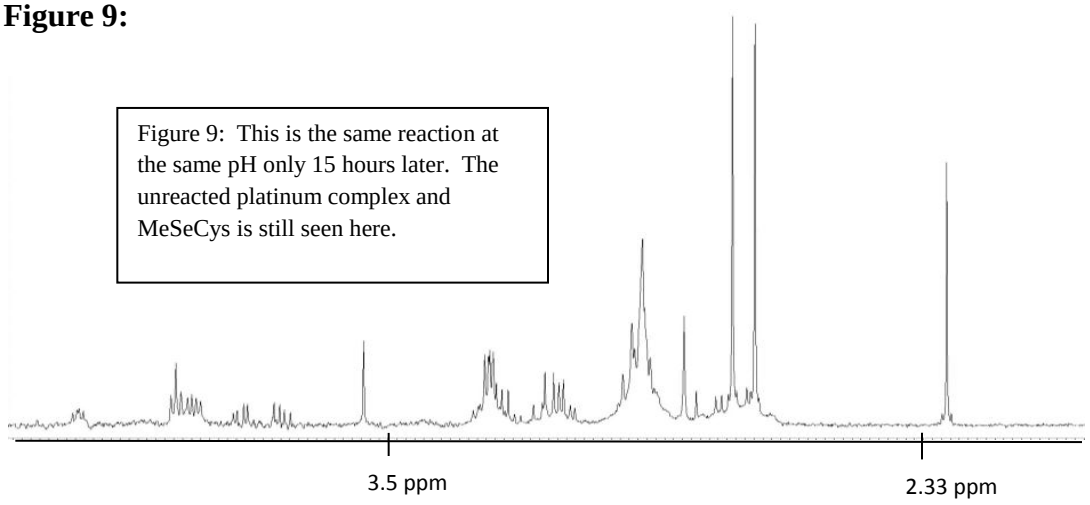


Figure 9:

Figure 9: This is the same reaction at the same pH only 15 hours later. The unreacted platinum complex and MeSeCys is still seen here.



CHAPTER 4

DISCUSSION

Part of the purpose of these experiments was to see the effects that bulky platinum (II) compounds have when reacted with selenium-containing amino acids, in particular selenomethionine and selenocysteine. Due to being unable to actually purchase selenocysteine, methylselenocysteine (MeSeCys) and selenocystine (SeCys) were purchased. Initially it was wanted to start working with selenocysteine and reacting it with the platinum compounds, methylselenocysteine was used first. After this reaction was completed, the obtained results showed that two isomers were being formed, which was caused from there being a chloride ion in solution from the methylselenocysteine. This gave the idea to try sodium chloride with selenomethionine and the platinum compound to see if any steric effects or isomers were forming. Back to the selenocysteine idea, it was ended up that using selenocystine for the next reactions with the Pt(en) compound. After not getting very good results, next the methylselenocysteine with the Pt(en) compound was repeated; this also did not help come to any conclusion for the seleocysteine project.

When the MeSeCys reacted with the $Pt(Me_4en)$ compound, the chloride adduct and MeSeCys chelate signals had formed in the NMR spectrum. Over time the chloride adducts begin to form relatively proportional to the MeSeCys chelates being formed.

Eventually the chloride adducts dissociate from the platinum compound, which was shown by the NMR spectra, leaving with only two peaks for both isomers of the chelates being formed. This information explains that the chloride ions from the MeSeCys are removed from the amino acid to form a monoadduct with the Pt(Me_4en) complex. Also, both isomers of the MeSeCys chelates form at relatively proportional rates. When this showed that both isomers for the chelates were being formed, it was decided to try a similar reaction with selenomethionine and the same platinum compound.

For the similar reaction sodium chloride was incorporated two different ways into the solution. The Pt(Me_4en) complex was mixed with sodium chloride and after approximately 30 minutes there were peaks that were associated with the formation of $[Pt(Me_4en)(D_2O)Cl]^+$. There were two singlets formed at 2.84 ppm and 2.85 ppm that were essentially assigned to the two methyl groups of the Me_4en ligand. Then the selenomethionine was added to the solution, this gave peaks at 2.38 and 2.39 ppm, that corresponds to the *Se*-CH_3 groups formed by the product $[Pt(Me_4en)(SeMet\text{-}Se)Cl]^+$. After approximately 5 days, these monoadducts form to Se-N chelates giving $[Pt(Me_4en)(SeMet\text{-}Se,N)^+$. It also noted that with the isomers that were formed, one was forming at a relatively fast rate compared to the other. The *R* isomer was formed first and slowly the *S* isomer was formed. The methyl group from the SeMet is sterically hindered from the methyl groups of the platinum complex. When the *S* isomer is formed, there is less room for the methyl group to exist, thus it wouldn't be expected to see this isomer form particularly fast. When the *R* isomer is formed, there isn't anything sterically hindering the methyl group, thus it is easier and faster in forming the chelate. Also, the SeMet was mixed with sodium chloride and allowed to mix for 30 minutes, then adding

the platinum compound. With this experiment, the same results are seen as did with the previous way the solution was mixed. The *R* isomer forms first and then slowly the *S* isomer forms. However, with this mixture the monoadduct is not seen like that its formed in the previous experiment. Finally, it was finally obtained, a great NMR spectrum for the isomers involved in the chelate formation, eventually it was decided to try the SeCys with a different platinum compound.

Using selenocystine with the Pt(en) complex gave very interesting results, however, it has not been able to classify peaks and determine what type of products that are obtained. With these experiments, a few things were noted, like color change and precipitate being formed in solution. For example, both of the solutions that were at a final pH of 7, were both translucent, but had a color of yellow. But with both solutions that had final pH's of 3 were both translucent, colorless. 2 days later a color change in solution were seen, both solutions that were initially yellow went colorless and formed a moderate amount of black precipitate. Both solutions that were initially colorless, had changed to a pale yellow color and also had black precipitate in solution that had formed 2 days later.

NMR data together was put together to obtain some information to help determine what is being formed in solution. Both concentrations of solutions at a pH of 3 showed on NMR data that the 'en' ligand dissociated from the platinum complex; while at a pH of 7, it did not. This can be concluded that the 'en' ligand is capable of coming off, but only at a low pH. There were also other peaks noted in the NMR spectra that were relatively similar to one another, but also one of the pH solutions would have a peak somewhere that the other did not. For example, at a pH of 7, peaks at 2.8 ppm and 2.2

ppm are seen, but it's not seen in the pH 3 solution NMR spectrum. But at both pH's there are peaks at 2.5 and 2.6 ppm. At the time of writing this thesis, we have not come up with any conclusions for what these peaks are. We have tried other experiments to try to see if we can figure it out, we have also done liquid/chromatography mass spectrometry, but have also not come up with any solid answers yet.

This experiment was completed using one solution at a pH of 3 and the other at 7, both solutions was also mixed as one platinum molecule for every one selenium molecule. Here, the pH 3 solution shows the 'en' ligand comes off again, but does not at pH 7. Inconclusive results were obtained for this experiment as well. We have not completed any mass spectrometry at the time of the thesis being written.

It is important however, that with the latter of the experiments we make a note that the 'en' ligand only comes off here with a low pH of around 3, rather than at a higher pH of around 7. For prior experiments in this thesis, we have finally captured the isomers being formed of the chelates for SeMet and Me_4en, and the monoadducts that formed. Prior to this experiment it was noted that the monoadducts were formed so fast, it had not been captured, and there was no information on the stereochemistry formed from these reactants as well. Now that we have made a few conclusions with a few bulky platinum compounds and these two selenium containing amino acids, we will continue to further explore other bulky platinum compounds with the amino acids. We will also further investigate our SeCys and Pt(en) compounds and get some finite results and answers and publish them.

Platinum compounds have been used alone and also in conjunction with other anti-cancer agents to fight the battle of cancer. Since platinum has a high affinity for sulfur containing amino acids and proteins, it also has an even higher affinity for selenium containing amino acids and proteins. These results produced in this research have contributed to the cancer research being conducted, it also gives insight into further research areas to explore.

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