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Relative Reaction Rates of the Amino Acids Cysteine, Methionine, and Histidine with Analogs of the Anti-Cancer Drug Cisplatin

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RELATIVE REACTION RATES OF THE AMINO ACIDS CYSTEINE,
METHIONINE, AND HISTIDINE WITH ANALOGS OF THE ANTI-CANCER DRUG
CISPLATIN

A Capstone Experience/Thesis Project

Presented in Partial Fulfillment of the Requirements for

the Degree Bachelor of Science with

Honors College Graduate Distinction at Western Kentucky University

By:

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2015

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2015

ABSTRACT

We are studying the reaction of analogs of the anticancer drug cisplatin with amino acids that differ in size and shape. The reaction of cisplatin with proteins likely precedes reaction with DNA in the body, forming a variety of products that may be toxic to the human body. The size and shape of the platinum(II) complexes often affects the rate of reaction with proteins, more so than with DNA. In this study, triamine cisplatin analogs are reacted with the amino acids cysteine, methionine, and histidine simultaneously. These reactions are monitored by NMR spectroscopy. The effect of the bulk of the ligand and the pH under which the reaction occurs was explored. It is seen that the bulkier $[\text{Pt}(\text{Me}_5\text{dien})(\text{NO}_3)]^+$ complex prefers to coordinate with N-Acetylcysteine than L-methionine or L-histidine. When the pH was raised from 4 to 7, the coordination to the platinum complex and N-AcCys occurred at a much faster rate.

Keywords: Cisplatin, Cysteine, Methionine, Histidine, Anticancer, Nuclear Magnetic Resonance, Chemistry, Medicinal-Pharmaceutical Chemistry

Dedicated to everyone that has helped me throughout my undergraduate career: friends,
family, professors, and the Gatton Academy.

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This project would not have been possible without the help and support of everyone I have met during my undergraduate career at Western Kentucky University. I would first like to thank my project advisor, Dr. Kevin Williams, for supporting me and helping me through all of life challenges, from coursework to making personal life decisions. Our conversations that started off about chemistry and ending with a rant about football are some of my favorite. I would also like to thank the many professors in the Chemistry Department that have encouraged me to pursue a future career in this field: Dr. Dahl, Dr. Maddox, Dr. Pesterfield, and Dr. Nee. I would not have made it this far without all of your support and guidance.

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CHAPTER 1

INTRODUCTION

Cancer is a group of diseases characterized by uncontrollable growth and spread of abnormal cells (1). Approximately 13.7 million people in the United States were living with a history of cancer, as of January of 2012, and an estimated 1,665,540 new cases were to be diagnosed in 2014 (1). Given these statistics, it comes as no surprise that treatments for cancer is among the top areas of scientific research. The current treatments of cancer include surgery, radiation, hormone therapy, immune therapy, targeted therapy, and chemotherapy (2). However, as with all treatments and medications, there are many side effects, such as toxicity and resistance, and research aims to decrease these effects while also increasing their effectiveness to treat the disease. One popular area of anticancer medicine research revolves around platinum (II) - containing compounds. Current FDA-approved treatments of this kind include cisplatin, carboplatin, and oxaliplatin; these are some of the most widely used chemotherapy drugs. Unfortunately, these medicines have harsh side effects like many other drugs.

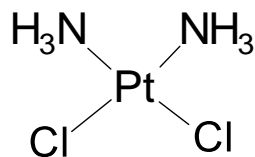


Figure 1: Structure of *cis*-diamminedichloroplatinum(II), or cisplatin

The anti-cancer activity of the platinum(II) compounds was first discovered, by accident, by the Rosenberg group at Michigan State University in 1965. Rosenberg saw that the electrolysis of platinum electrodes created a platinum complex that inhibited the cell division of *Escherichia coli* (*E. coli*) bacteria while the cell growth was not bothered (3). The platinum complex generated was found to be *cis*-diamminedichloroplatinum(II), or cisplatin. It was later tested on sarcomas in rats and it was found that the complex was effective in reducing the mass of the tumors (4). This finding led to other conformation testing in various cancer cell lines and led to its approval for clinical use by the FDA in 1978 (5).

Cisplatin is most effective on testicular and ovarian cancers, two forms of cancer that yielded a high fatality rate prior to the discovery of the anticancer activity of the platinum(II) complexes. Prior to 1970, testicular cancer killed over 90% of those diagnosed (5). After the introduction of cisplatin to the treatment regimen in 1978, over 80% of patients survived the disease (5). Although the drug is very successful in treating these cancers it also has many harsh side effects. These include, but are not limited to, nephrotoxicity, ototoxicity, neurotoxicity, vomiting, and seizures (2). It is apparent that cisplatin causes programmed cell death throughout the body, not just in cancer cells. Due to the severe side effects of the drug, studies have been conducted to improve the structure and functionality of the platinum compound to reduce these ill-effects and improve its anticancer activity. In order to determine what causes the harsh side effects, it is necessary to understand how the drug works in the body.

Cisplatin, administered intravenously, in its dichloro form is highly stable and unreactive in areas of high chlorine concentrations, such as blood plasma where the concentration is greater than 100 mM (6). The dichloro form then enters the cell, recently proposed to be mediated through the copper transporter CTR1, into the relatively low chloride concentrated environment of cytoplasm (7). At this point in cisplatin's journey, the chlorines are displaced via aquation (shown in Figure 2), yielding a highly reactive electrophile whose ionic charge might prevent it from exiting the cell (6). This reactive species can bind covalently to a variety of macromolecules, where DNA is the most important, but not only, target of cisplatin.

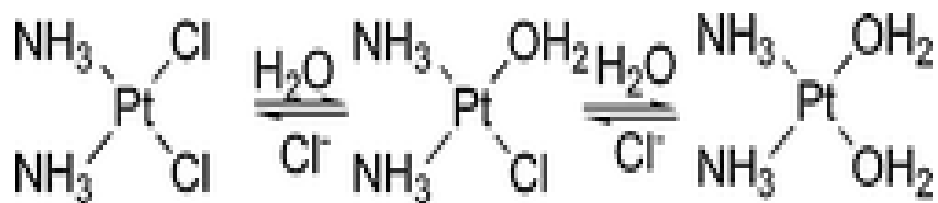


Figure 2: Shows the aquation of cisplatin once inside the cell.

The anticancer activity of cisplatin is attributed to adducts the active aquated form makes with the nucleobases of DNA. Cisplatin has a preference to bind at the N7 position of the guanine residue on the double helix (2). The 1,2 – intrastrand crosslink formed distorts and unwinds the duplex helix of the DNA, which blocks DNA replication and transcription, resulting in programmed cell death, or apoptosis (6). There are three other types of adducts that can be formed between DNA and cisplatin: monoadduct, interstrand crosslink, and protein-DNA crosslink (8). Typically a monoadduct is initially formed and

the complex then goes on to form the other types of adducts shown in Figure 3 (8). The interstrand crosslink and protein-DNA crosslink are less common but could contribute to cytotoxicity as they also impede DNA replication and transcription (8).

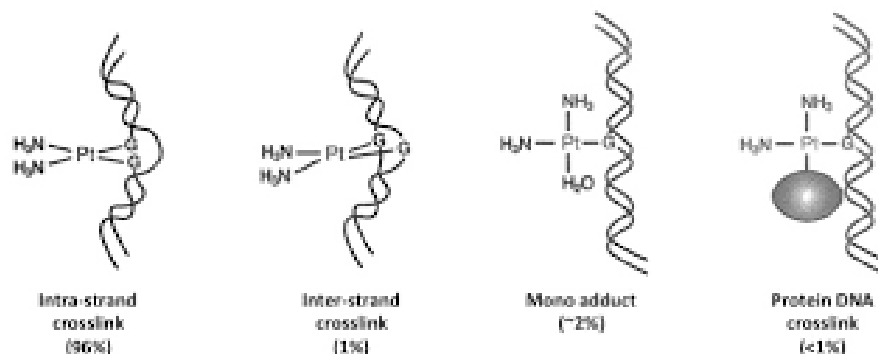


Figure 3: Demonstrates the four Pt-DNA adducts that can be formed: Intrastrand crosslink, interstrand crosslink, monoadduct, and protein-DNA crosslink

However, cisplatin does not only form adducts with DNA but also with proteins and other macromolecules. It is these adducts that are thought to be the source of the drug's toxicity and resistance (9). While the formation of the Pt-DNA adducts requires the aquation of the platinum compound, the Pt-protein adducts can occur in the dichloro form, resulting in a preference of platinum(II) for proteins and related macromolecules (10). It has been discovered that 24 hours after the cisplatin is administered to the patient, 65-98% of the compound has formed adducts with proteins (9). It is therefore important to understand the reaction of platinum compounds with DNA and proteins and what factors might affect the reaction with each.

In previous studies, cisplatin and the other platinum(II) compounds have shown a preference for sulfur containing groups (9). This preference leads to cisplatin forming

adducts at cysteine or methionine residues within proteins. These amino acids are the only two that contain a sulfur side chain. However, cisplatin also shows a preference for histidine, a non-sulfur containing amino acid, though it is less significant (9). This preference can be explained using Hard-Soft Acid Base (HSAB) theory. An atom or molecule can be described as Lewis acid or base. A Lewis acid is defined as a species with a tendency to accept an electron pair while a Lewis base is a species with a tendency to donate an electron pair (11). In the reaction chemistry of platinum(II) complexes and the biological macromolecules they favor, the platinum(II) is the acid and the sulfur, nitrogen, or oxygen atom it binds with is the base.

The Hard-Soft Acid Base theory places these acids and bases into categories of hard and soft. Polarizable acids and bases are characterized as soft while nonpolarizable species are designated as hard (11). The distinction between hard and soft heavily relies on the degree to which a molecule or ion is distorted by interaction with other molecules or ions (11). In line with this theory, platinum(II) is considered a soft acid, due to its large size and low charge. For bases, the trend is easy to see. Within a group, the atoms become softer going down the group (11). By this we can say sulfur is softer than oxygen because it is more polarizable due to its larger size with more electrons (11). According to Ralph Pearson, who proposed HSAB theory, hard acids prefer to bind to hard bases, and soft acids prefer to bind to soft bases (11). Through this, the preference for the sulfur containing side chains of cysteine and methionine can be understood. Because platinum(II) is a soft acid and sulfur is a soft base, they will have a higher affinity to bind together due to the theory of HSABs.

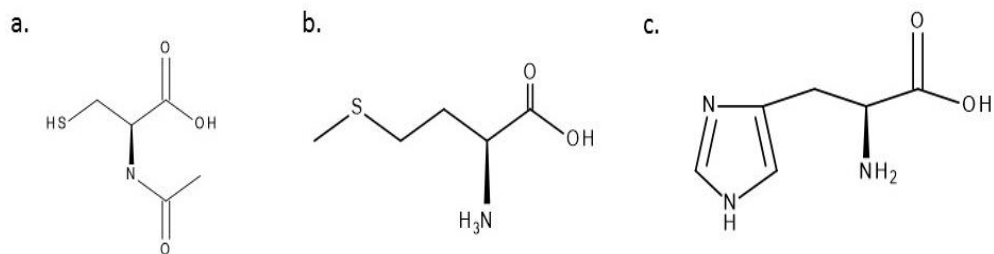


Figure 4: Structures of the amino acids: a. N-AcetylCysteine b. L-Methionine
c. L-Histidine

In order to decrease the toxicity of cisplatin and increase its activity, the ligand sphere around the central atom has been changed (2). This manipulation of the ligand has led to the creation of the second and third generation platinum(II) chemotherapy drugs, carboplatin [cis-diammine-1,1-cyclobutanedicarboxylatoplatinum (II)] and oxaliplatin respectively (2). Both of these complexes show an equivalent or better activity than cisplatin and less toxicity than the first platinum(II) chemotherapy drug (2). This project looks at the reactivity of two analogs of cisplatin (Figure 5): diethylenetriamineplatinum(II) dinitrate, $[\text{Pt}(\text{dien})(\text{NO}_3)]^+$, and pentamethyldiethyletriameplatinum(II) dinitrate, $[\text{Pt}(\text{Me}_5\text{dien})(\text{NO}_3)]^+$.

Both of these derivatives have a similar structure, as can be seen in Figure 5. The main difference between these two is the size of the ligand chelated to the central platinum atom. The Me_5dien ligand has the same basic ligand structure as the dien ligand but with five methyl groups attached at the nitrogen atoms. This creates a much bulkier ligand, which has been proven to slow down reaction with nucleobases and amino acids alike. In previous studies it has been shown that the $[\text{Pt}(\text{Me}_5\text{dien})(\text{NO}_3)]^+$ complex has a higher affinity to bind to the nucleobase guanine than with the amino acid methionine

whereas $[\text{Pt}(\text{dien})(\text{NO}_3)]^+$ showed the opposite trend (12). Knowing this, it is important to also determine which amino acid is favored by the Me_5dien platinum complex. This project will demonstrate the reaction of the two derivatives of cisplatin shown in Figure 5 with a competition of the amino acids cysteine, methionine, and histidine in order to determine which amino acid the complexes prefer, if there is a preference.

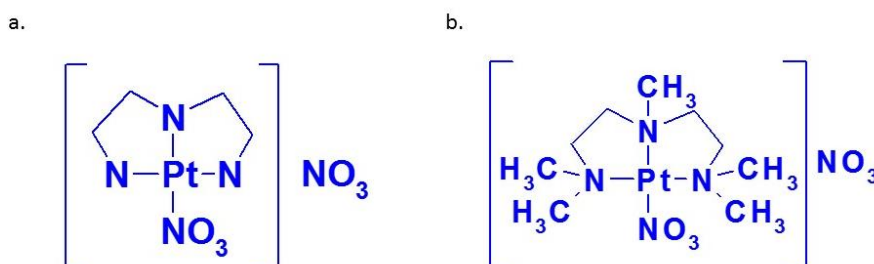


Figure 5: Structures of the cisplatin derivatives:

- a. diethylenetriamineplatinum(II) dinitrate $[\text{Pt}(\text{dien})(\text{NO}_3)_2]$
- b. pentamethyldiethylenetriamineplatinum(II) dinitrate $[\text{Pt}(\text{Me}_5\text{dien})(\text{NO}_3)_2]$

In addition to exploring the affinity of the complexes for different amino acids, this project will also determine the effect of pH upon the reaction. Previous studies have examined the reaction of platinum(II) compounds with DNA nucleobases and amino acids at the pH of 4 (9, 13). At this pH, the amino acids cysteine, methionine, and histidine remain in their protonated state. This is particularly important for cysteine. Raising the pH to 7, the biological pH, alters the charge of the molecule. The sulfur group of cysteine becomes deprotonated due to its pK_a of around 8.0, as seen in Figure 6, creating the more reactive form of the amino acid (13). This is thought to affect its reaction rate relative to that of methionine at the same pH. The same was thought to

occur for the histidine amino acid, as it also deprotonates at pH 7. However, previous studies have shown that the change in pH has little bearing on the reactivity of histidine

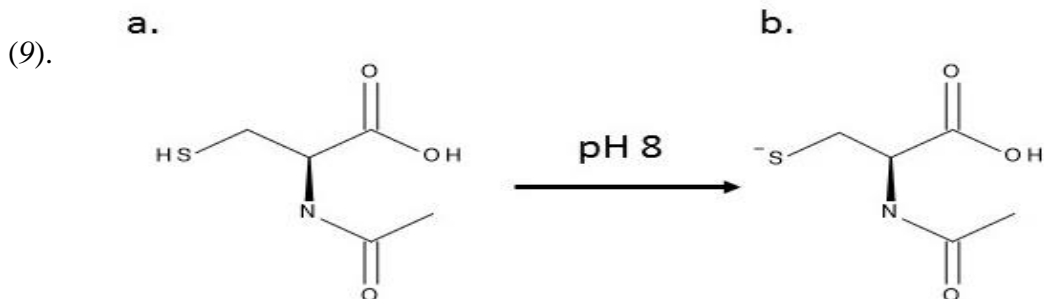


Figure 6: The deprotonation of N-Acetylcysteine from a.) pH 4 to b.) pH 8.

In addition to the change in amino acid structure, the platinum complexes form dimers at a higher pH. These dimers have an altered reactivity from the platinum structure at pH 4. This project explores the effect of higher pH on the reaction of the triamine compounds with the three amino acids. The relative rates of reaction between cysteine, methionine, and histidine are examined at the pHs of 4 and 7 to determine if the change in the cysteine amino acid affects the rate of reaction.

The experiments involved in this project were monitored using Nuclear Magnetic Resonance, or NMR, spectroscopy. NMR spectroscopy is a technique used to determine the structure of a molecule or compound. This instrumentation places a sample into a static magnetic field which is then exposed to a secondary oscillating magnetic field. In this project ^1H NMR spectroscopy is essential to determine the formation of products during the reaction. In ^1H NMR, a peak is shown on the spectrum based on the environment around each unique hydrogen atom in the sample. The atoms surrounding a hydrogen atom affect the shift of the peaks down the spectrum. Spectra taken over a

period of time can be used to determine the formation of products and their relative abundance in the sample. Over time, peaks that are not characteristic of the reactants are labeled as product peaks. This demonstrates the change in the chemical structure of the reactants as the reaction moves toward the products. Following the development of these product peaks can show which product is favored and the rate of the reaction.

CHAPTER 2

EXPERIMENTAL METHODS

Synthesis of Pt(Me₅dien)(NO₃)₂. In order to synthesize [Pt(Me₅dien)I]₂ (Pt₂I₆), which is needed before forming the nitrate and adapted from a previous method in Romeo et al., 0.5 g of potassium tetrachloroplatinate was dissolved in 5 mL of deionized water in an amber vial (13). 82 g of potassium iodide was added and the mixture was stirred for 5 minutes at 50°C. Once the 5 minutes had passed, 250 µL of pentamethyldiethylenetriamine was added. The solution was stirred at 50°C for one hour, after which the green precipitate was separated via gravitational filtration. This process yielded 506.6 mg of the iodide, which was allowed to dry over 2 nights to reduce moisture left in the product.

300 mg of the dried Pt(Me₅dien)I₂ was added to 20 mL of deionized water in an amber vial. 98.5 mg of silver nitrate was added to the vial and the mixture was stirred overnight. The product was filtered out of the solution using a syringe filter and collected in a round bottom flask. The remaining product was then isolated using a rotary evaporator yielding 97.8 mg of Pt(Me₅dien)(NO₃)₂. A small sample of the product was dissolved in 1 mL of deuterium oxide and the identity was confirmed by ¹H NMR using a 500 MHz JEOL Eclipse instrument.

Preparation of Pt(Me₅dien)(NO₃)₂ and N-AcCys, L-Met, L-His Solutions.

Both solutions were made at 20 mmol concentrations. For the platinum complex solution, 9.8 mg of Pt(Me₅dien)(NO₃)₂ was dissolved in 1.0 mL of deuterium oxide. The pH of the solution was adjusted to either 4 or 7, depending on the reaction, using deuterated nitric acid and sodium deuterioxide solutions. The amino acid solution was made by mixing 3.1 mg of L-His, 3.3 mg of N-AcCys, and 3.0 mg L-Met in a 4 mL vial and dissolved in 1 mL deuterium oxide. The pH was adjusted in the same manner as the platinum solution.

Reaction of Pt(Me₅dien)(NO₃)₂ and N-AcCys, L-Met, L-His at pH 4. The platinum solution and the amino acid solution were both adjusted to pH 4 using the procedure detailed above. 300 μL of each solution was added to an NMR tube and mixed together. Kinetics of the reaction was monitored using a 500 MHz JEOL Eclipse instrument over a period of 12 hours, taking an acquisition scan every 15 min yielding 48 data points.

Reaction of Pt(Me₅dien)(NO₃)₂ and N-AcCys, L-Met, L-His at pH 7. The platinum solution and the amino acid solution were both adjusted to pH 7 using the procedure detailed above. 300 μL of each solution was added to an NMR tube and mixed together. Kinetics of the reaction was monitored using a 500 MHz JEOL Eclipse instrument over a period of 12 hours, taking an acquisition scan every 15 min again yielding 48 data points.

CHAPTER 3

RESULTS

Synthesis of $[\text{Pt}(\text{Me}_5\text{dien})(\text{NO}_3)]^+$: A ^1H NMR spectrum was taken in order to confirm the identity of the cisplatin analog (Figure 7). The compound is characterized by three major peaks at 2.61, 2.78, and 2.91 ppm (12,14). It is this synthesized compound that is used for all of the following reactions.

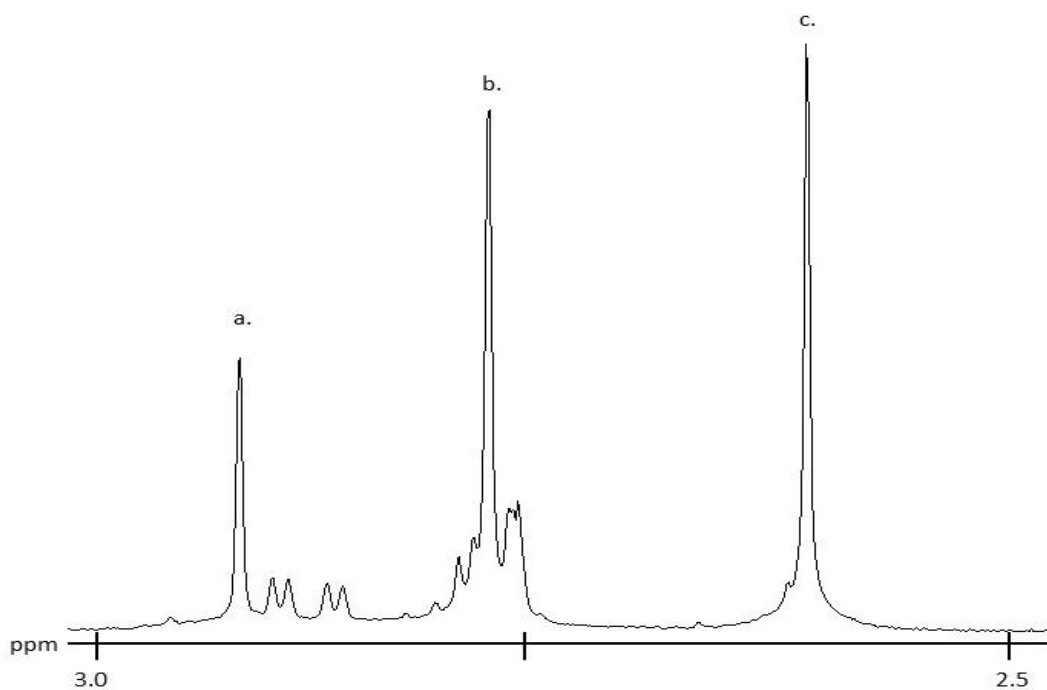


Figure 7: ^1H NMR spectrum of the synthesized $[\text{Pt}(\text{Me}_5\text{dien})(\text{NO}_3)]^+$ compound. It is characterized by three major signals: a.) 2.91ppm, b.) 2.78ppm, c.)2.61ppm.

Reaction of Pt(Mesdien) with N-AcCys, His, Met at pH 4: One equivalent of the amino acid mixture of N-Acetylcysteine (N-AcCys), L- Methionine (L-Met), and L- Histidine (L-His) was added to $[\text{Pt}(\text{Me}_5\text{dien})(\text{NO}_3)]^+$ and monitored via ^1H NMR continuously over the course of 12 hours. Analysis of certain spectral peaks is vital in monitoring the reaction. Figure 8 demonstrates which hydrogens on each amino acid is being monitored in the spectra. The peaks of interest for each amino acid are as follows: the S-CH₃ of L-Met, the acetyl hydrogen of N-AcCys, and the imidazole hydrogens of L-His. These peaks were chosen due to their appearance as a singlet that is clearly separated from other signals. When the amino acid coordinates to the platinum, the new product peak will be shifted due to the new environment around the hydrogen. The growth of these peaks, relative to that of the others, can be used to determine which amino acid is favored by the platinum complex.

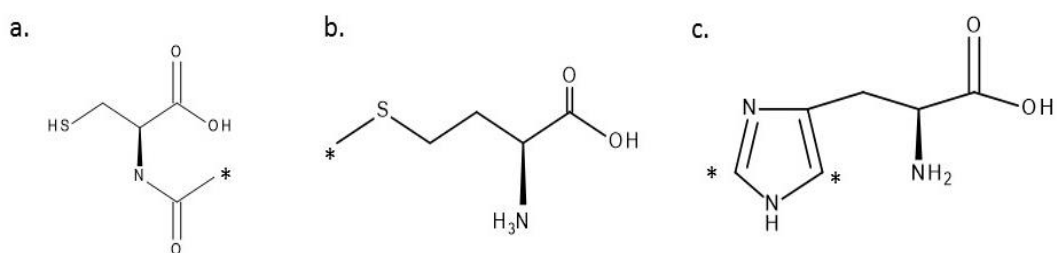


Figure 8: The hydrogen peaks of interest are designated with an asterisk for: a.) N-AcCys b.) L-Met c.) L-His

The spectra showing the ^1H NMR signals for the reaction of L-His with $[\text{Pt}(\text{Me}_5\text{dien})(\text{NO}_3)]^+$ at pH 4 are shown in Figure 9. The spectra were collected at the initiation of the reaction and 5 days after that point. If the platinum complex coordinated with the amino acid, two new singlets would form on the spectra near the original peaks.

However, as can be seen in Figure 9, negligible reaction occurred with L-His, signified by the presence of only the two original signals.

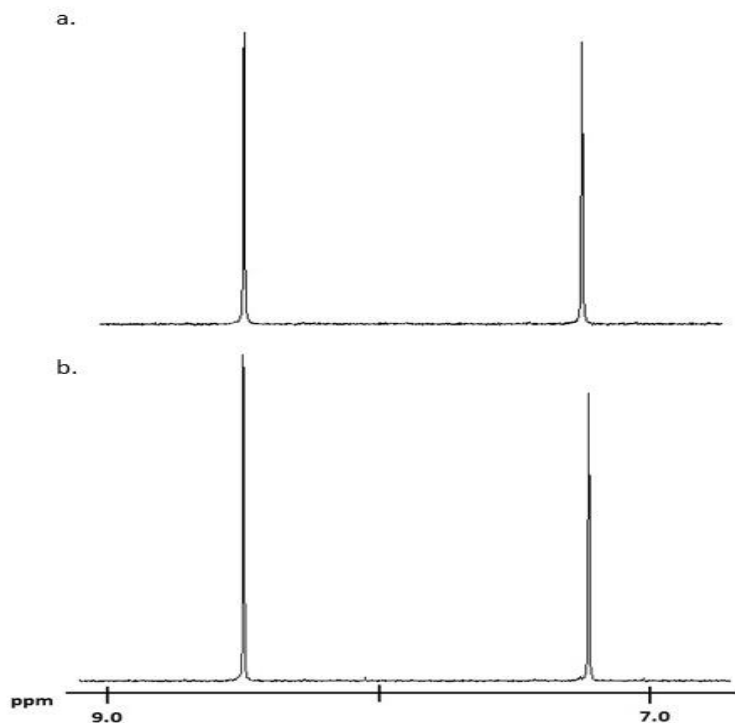


Figure 9: ^1H NMR spectra showing the histidine signals at a.) 15 min b.) 12 hours for the reaction at pH 4

The ^1H NMR spectra showing the progression of reaction of the platinum complex with L-Met and N-AcCys are represented in Figure 10. The first spectrum in the figure demonstrates the reaction after 15 min, representing the reaction at its beginning. The three characteristic platinum complex singlets are seen in the same positions as in Figure 7. The unreacted N-AcCys peak of interest, representing the acetyl group of the molecule, is seen as a singlet at 1.92 ppm. A singlet at 1.97 ppm represents the S-CH₃ group of the unreacted L-Met. Throughout the reaction, new singlets that are shifted from these positions will form, representing the coordination complex between the sulfur atom of the amino acid and cisplatin derivative.

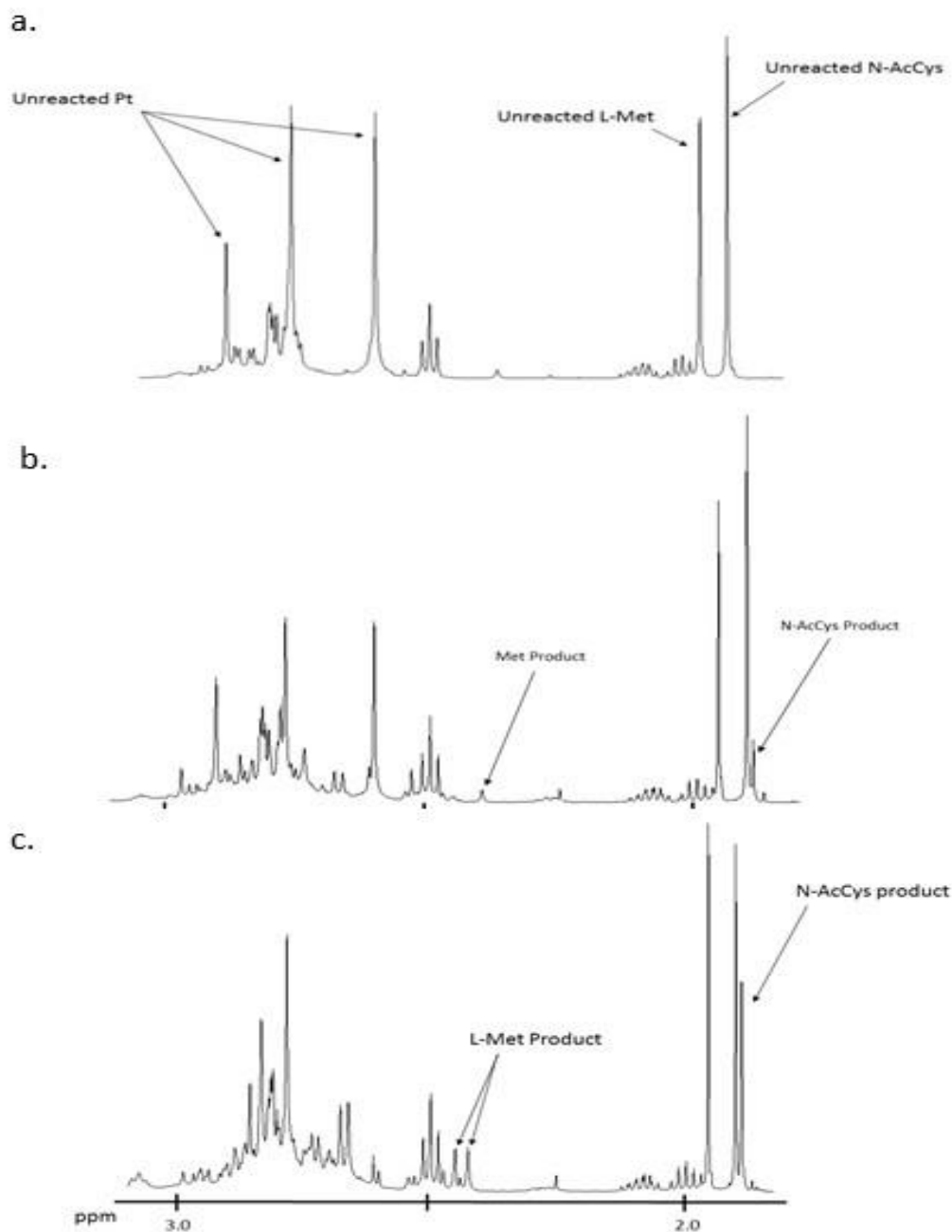


Figure 10: ^1H NMR spectra of the reaction of $[\text{Pt}(\text{Me}_3\text{dien})(\text{NO}_3)]^+$ with N-AcCys and L-Met at pH 4. a.) Initial spectrum taken of the reaction after 15 min. b.) Spectrum of reaction after 12 hours, where the N-AcCys product at 1.89ppm can be seen, as well as the L-Met product at 2.43 ppm. c.) Spectrum of reaction after 5 days, where a preference for N-AcCys is seen, and the development of the L-Met product twin singlets at 2.42 and 2.45ppm.

In the second spectrum (b) of Figure 10, the state of the reaction after 12 hours can be seen. Two new resonances appear on the spectrum, each representing the coordination of one of the amino acids with the platinum complex. A new singlet at 1.89 ppm represents the coordination of N-AcCys with Pt(Me₅dien). Due to the new environment on the N-AcCys molecule, the singlet representing the acetyl group was shifted upfield, or to the right, from the original, unreacted peak. Its relatively small height compared to the unreacted singlet shows that little reaction has occurred by this time in the reaction. A second singlet appears around 2.43 ppm, which represents the coordination at the sulfur atom of L-Met. This peak is shifted more significantly upfield, or to the left, of the original peak of the unreacted L-Met than the N-AcCys product was shifted due to the proximity of the platinum complex to the hydrogen of interest. On N-AcCys, the platinum coordinates to a position that is far from the hydrogen of interest while on L-Met, the platinum coordinates to the atom that the hydrogen is bonded to. This proximity causes a much greater shift of the S-CH₃ signal. This L-Met product singlet is much smaller in relation to the unreacted peak than the N-AcCys product is to its counterpart, signifying that N-AcCys is preferred by the platinum complex.

The reaction was revisited 5 days after its initiation in order to determine if one amino acid was significantly preferred, as both product peaks were small after 12 hours. This spectrum is shown in Figure 10 (c). In this spectrum, the N-AcCys product peak has grown significantly more in comparison to the L-Met product peak. The most significant change in the L-Met product is the formation of two singlets instead of just one singlet as seen at 12 hours into the reaction. This formation is due to the slow interchange in the

isomerization of the complex. The L-Met sulfur, after the coordination of the platinum, becomes chiral. These two singlets (Figure 11) represent the diastereomers that are a result of this chirality. This phenomenon has been seen in a previous study between methionine and platinum (II) complexes (15).

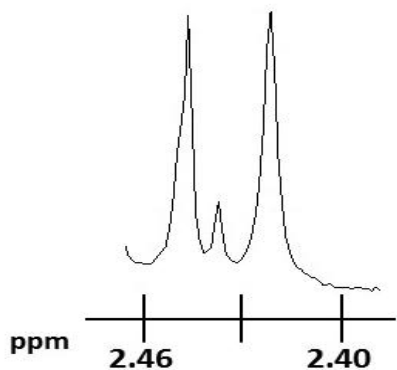


Figure 11: The twin singlets between 2.40 ppm and 2.46 ppm represent the chirality about the S-atom of the L-Met product. Taken from the spectrum of 5 days after reaction initiation.

Reaction of Pt(Mesdien) with N-AcCys, His, Met at pH 7: As seen in the reaction at pH 4, there was negligible reaction with L-His 12 hours after the initiation of reaction. L-His is deprotonated at pH 6, creating a more reactive form of the amino acid. The spectra taken at the beginning of reaction and after 12 hours is shown in Figure 12. There is no development of new resonances, signifying that no L-His product was formed. This shows that even the deprotonated, more reactive form of L-His is not preferred by the platinum complex in relation to the sulfur-containing L-Met and N-AcCys.

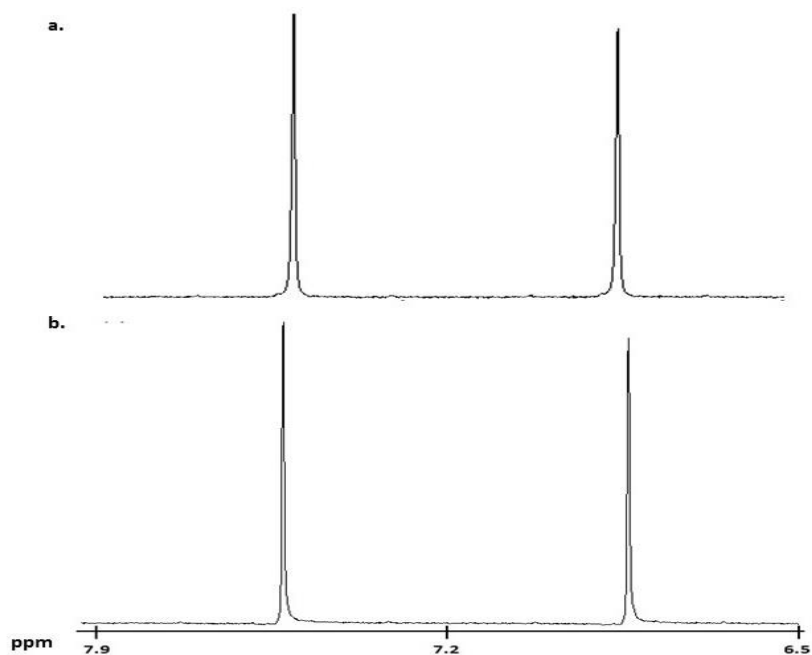


Figure 12: ^1H NMR spectra showing the histidine signals at a.) 15 min b.) 12 hours for the reaction at pH 7

The ^1H NMR spectra taken of the reaction at pH 7 is shown in Figure 13. The initiation of reaction is shown in the first spectrum of the figure, as with the reaction at pH 4. However, unlike the previous reaction, the sample was not revisited several days later. Figure 13a shows the three characteristic signals for the platinum complex as well as the unreacted amino acid peaks seen in the pH 4 spectrum. Figure 13b represents the reaction after 12 hours. In this spectrum, a significant N-AcCys product singlet can be seen at 1.89 ppm. The height of the product is close to the same height as that of the unreacted N-AcCys. This signifies that there is roughly the same amount of unreacted N-AcCys as product. There is also a small L-Met product peak around 2.43 ppm. In comparison to the N-AcCys product, the L-Met product is barely formed, suggesting that N-AcCys is highly preferred at pH 7.

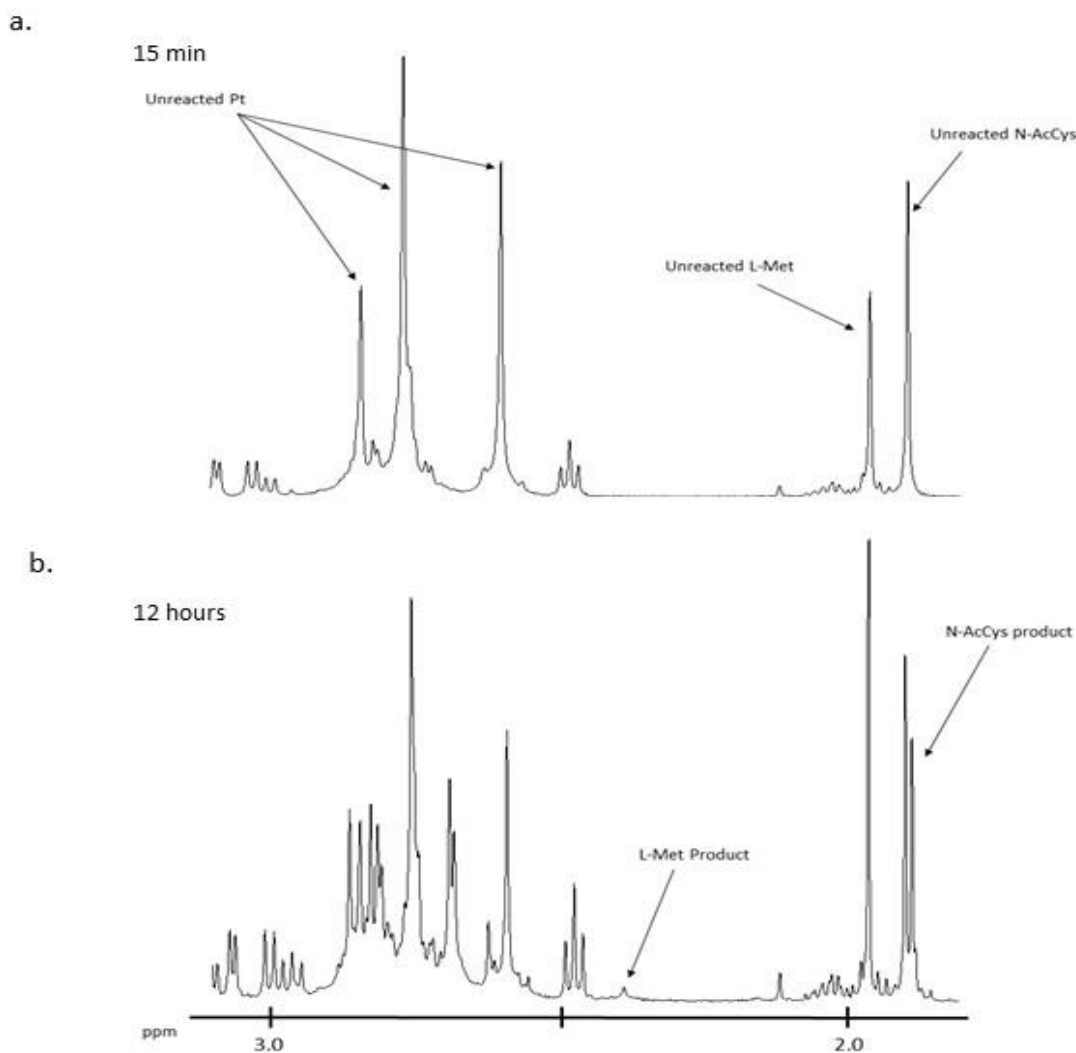


Figure 13: ^1H NMR spectra of the reaction of $[\text{Pt}(\text{Me}_5\text{dien})(\text{NO}_3)]^+$ with N-AcCys and L-Met at pH 7. a.) Initial spectrum taken of the reaction after 15 min. b.) Spectrum of reaction after 12 hours, where the N-AcCys product at 1.89ppm can be seen, as well as the L-Met product at 2.43 ppm.

Figure 14 shows a comparison of the 12 hour spectra of reaction at pH 4 and 7. As can be seen in the spectra, the N-AcCys product forms much faster at pH 7 than pH 4. In comparison, the L-Met product appears to form at roughly the same rate. Very little product is seen in either reaction. The L-Met product is represented by a single singlet

instead of the twin singlets seen in the pH 4 reaction at 5 days of reaction. However, the L-Met was seen as a singlet at that reaction's 12 hour mark. Since the pH 7 reaction was not revisited as the pH 4 was, the twin singlets could have formed after the 12 hour mark as they did at pH 4.

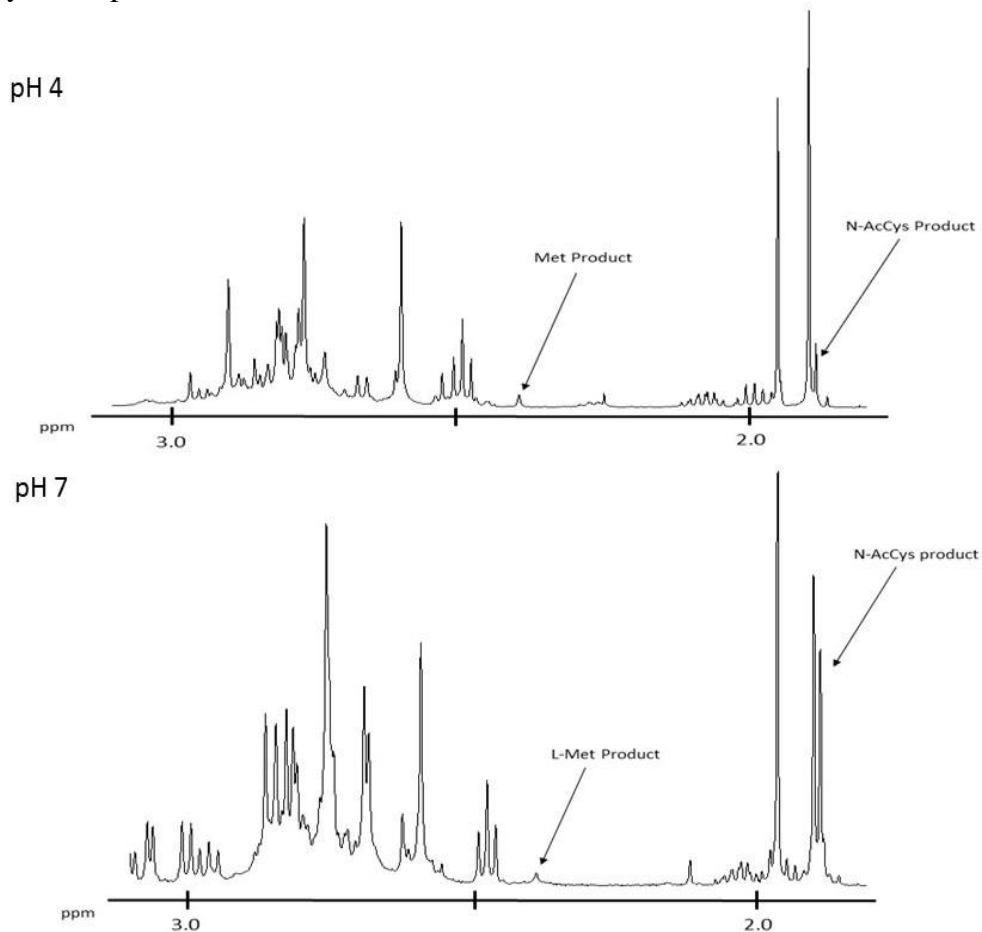


Figure 14: ¹H NMR spectra representing the pH 4 and 7 reactions at 12 hours into the reactions. The N-AcCys product forms at a much faster rate at pH 7 than pH 4, as seen by the more abundant signal.

CHAPTER 4

DISCUSSION

It has been shown in previous experiments that the cisplatin derivative of $[\text{Pt}(\text{Me}_5\text{dien})(\text{NO}_3)]^+$ has a higher affinity for the nucleobase guanine than the amino acid methionine (12). This project expands on this finding, looking at the cisplatin derivative's preference for a certain amino acid among the main targets of cysteine, methionine, and histidine. The effect of pH was also examined for the competition reaction, conducting the experiment at both pH 4 and pH 7. The pH within the body is 7, so it is important to understand how these platinum(II) compounds react with amino acids at this pH. Histidine has a pK_a of 6, meaning it is fully deprotonated at pH 7, while N-acetylcysteine has a pK_a of 8, where it will have some of the sample deprotonated. The deprotonated forms of these amino acids are more reactive and the effect of this increased reactivity is examined.

At both pH values, the reaction of L-His and the platinum complex $[\text{Pt}(\text{Me}_5\text{dien})(\text{NO}_3)]^+$ is negligible. In the spectra shown in Figures 8 and 12, there was no development of singlets that would represent an L-His product, even after several days of reaction. Although the L-His is deprotonated and therefore more reactive, the platinum (II) compound prefers the sulfur-containing amino acids methionine and cysteine. The platinum coordinates at a nitrogen group on the L-His which is not as soft of a base as

sulfur, which may explain the non-reactivity with histidine when in the presence of the sulfur groups of cysteine and methionine.

The reaction of $[\text{Pt}(\text{Me}_5\text{dien})(\text{NO}_3)]^+$ with N-AcCys and L-Met at pH 4 was monitored continuously over 12 hours and revisited after 5 days. After just 12 hours of reaction, there was a preference for N-AcCys, as seen in the more significant growth of its product signal compared to that of L-Met. However, both signals were small in comparison to the unreacted amino acid peaks, signifying that little reaction had occurred up to that point. Because of the small amount of reaction that had occurred by 12 hours into the reaction, it was revisited 5 days later. The spectrum taken at this point, shown in Figure 13, shows a much higher growth in the N-AcCys product signal than in the L-Met product. Based on this spectrum, it can be concluded that $[\text{Pt}(\text{Me}_5\text{dien})(\text{NO}_3)]^+$ has a much higher affinity for N-AcCys than L-Met.

Increasing the pH from 4 to 7 had a significant effect on the reactivity of N-AcCys with $[\text{Pt}(\text{Me}_5\text{dien})(\text{NO}_3)]^+$. After only 12 hours of reaction at pH 7, a higher ratio of N-AcCys product to unreacted N-AcCys can be seen than after 5 days of reaction at pH 4. This suggests that the higher pH allows for some of the cysteine to be deprotonated, creating the more reactive thiolate form of the amino acid. Given that the pK_a of cysteine is 8, raising the pH from 4 to 7 deprotonated a portion of the N-AcCys in the sample. In comparison to the protonated L-Met, the thiolate is much more reactive, coordinating much faster to the platinum complex while L-Met had roughly the same rate of reactivity. This finding is particularly important as the pH within the cell is also 7. This gives us a better idea as to where the platinum complex is likely to coordinate in the

body. Previous studies have shown that the $[\text{Pt}(\text{Me}_5\text{dien})(\text{NO}_3)]^+$ compound has a higher affinity for guanine than for methionine (12). However, since the cisplatin derivative shows a higher affinity for cysteine over methionine, the exploration into the compound's preference for cysteine or guanine will be important to determine the platinum complex's affinity for the amino acid or DNA nucleobase.

In the pH 4 reaction with the $[\text{Pt}(\text{Me}_5\text{dien})(\text{NO}_3)]^+$ complex, two singlets were seen for the L-Met product. This is likely due to the slow interconversion between the two isomers possible. When the platinum coordinates, the sulfur atom becomes chiral, giving two possibilities of isomers. At 12 hours into the reaction, only one singlet can be seen, suggesting that one isomer is faster to form than the other. When the pH 4 reaction was revisited after 5 days, the twin singlets could be seen, showing that both isomers eventually form, though one forms faster. It is possible that this also occurs at pH 7 though only one singlet could be seen at the 12 hour mark in the reaction. If this reaction was revisited after several days, as was the pH 4 reaction, this phenomenon might have been seen. However, there was significant reaction with N-AcCys at 12 hours, allowing us to clearly see which amino acid was preferred.

Platinum (II) compounds have long been used in conjunction with other drugs in the fight as against cancer. Because these compounds have high levels of toxicity and side effects, it is important to understand how these drugs work inside the body so they might be improved upon. Since platinum (II) has a high affinity for sulfur, common targets of these drugs are the sulfur-containing amino acids of cysteine and methionine. The coordination of cisplatin with amino acid residues of proteins is thought to be a

source of toxicity of the drug. Greater affinity for cysteine over other amino acids with bulkier platinum (II) compounds may lead to new methods of targeting and reacting within the cell. The results of this study into the amino acid preference of two triamine derivatives of cisplatin contributes to ongoing research of cancer treatments and gives insight into new areas of research with these compounds.

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