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Reactions of Cisplatin Analogs with Selenomethionine

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REACTIONS OF CISPLATIN ANALOGS WITH SELENOMETHIONINE

by

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Under the Direction of Dr. Kevin Williams

ABSTRACT

Cisplatin is a well-known anti-cancer drug that is effective, but also has some severe and unwanted side effects. Analogs of cisplatin were reacted with the nonstandard amino acid selenomethionine (SeMet), and the products were characterized by ^{1}H and ^{195}Pt NMR spectroscopy and HPLC. In previous studies, SeMet was found to react faster than methionine (Met) with a representative platinum complex ($Pt(dien)Cl_2$), therefore SeMet is said to react faster kinetically. Thus, while only a subset of proteins have selenium-containing amino acids, platinum complexes could target them kinetically. Cisplatin analogs with two leaving groups have also been studied. $Pt(Me_4en)(NO_3)_2$ (Me_4en = N,N,N',N'-tetramethylethylenediamine) reacts with SeMet to form a $[Pt(Me_4en)(SeMet\text{-}Se,N)]^+$ chelate regardless of the Pt:SeMet ratio. $Pt(en)(NO_3)_2$ (en = ethylenediammine) reacts to form three possible products, with the distribution dependent on the Pt:SeMet ratio and time.

INDEX WORDS: Cisplatin, Selenomethionine, Platinum(II) compounds, Hard-soft acid-base theory, *Trans* effect, Nuclear magnetic resonance

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DEDICATION

To Dr. Williams for being brave enough to take on another student with an Honors thesis project. Without him, I would not have been able to present this research at national meetings and gain experience with both poster and oral presentations. Much of my professional development is a direct result of his time, patience and support.

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INTRODUCTION

It was estimated by the American Cancer Society that approximately 10,701,000 people had a form of cancer in 2005, and the number of new cases each year continues to increase[1]. Odds are that a person will know one person, if not more, with cancer during their lifetime. And every person affected both directly and indirectly hopes that a cure will be found in time to help them. There are many promising treatments currently in use but still researchers are working to find more effective and less toxic treatments for a cancer cure. One such area of research is that involving the use of platinum(II) compounds for cancer treatments. In fact, Tour de France winner Lance Armstrong's cancer was successfully treated using such a compound[2]. Like many other developments in science, the discovery of cisplatin and its anti-cancer ability was an accidental event that occurred during another experiment involving bacteria. Since its discovery, many variations in the composition of the platinum(II) compounds have been developed and researched to improve its anti-cancer activity. The research in this project looked at two such compounds that are analogous to the structure of cisplatin.

The potential of cisplatin as an anticancer agent was stumbled upon around 1964. Barnett Rosenberg was exploring the effects of an electric field on bacterial growth. The experiment consisted of platinum electrodes in an aerobic solution of *Escherichia coli* with ammonium chloride.[3] Rosenberg was not looking for anticancer activity; he simply wanted to know what would happen to the bacteria in an electric field. The results of the experiment presented evidence that the cells were not participating in normal cellular division, but they did appear to be grow into long filaments 300 times the normal length. It was soon determined that the effects on the cells were not originating from the electrode alone, but an eletrolysis product in the solution, cis-$[Pt(NH_3)_2Cl_2]$.[4] This platinum compound was capable of compromising cell division without cell death, which made it a candidate for preventing tumor growth with minimal

damage done to cells of the host. Clinical trials were approved and began in the early 1970's, and cisplatin was FDA approved in 1979.

The chemistry of the platinum compound is important in that it results in specific behaviors that can be utilized when it is combined with other compounds. Platinum(II), the form in cisplatin, is an d^8 transition metal with square planar geometry at the platinum atom. The $Pt(NH_3)_2Cl_2$ compound under normal biological conditions has two ammine ligands and two chlorine ligands. Two arrangements are possible, a *cis* (Figure 1) and *trans* isomer. However, it was shown in several studies that only the *cis* isomer is effective as an anticancer drug. Many theories have been put forth as to why the *trans* isomer is not as effective, but no definite answer or combination of reasons has been verified through experimentation. Platinum is described as a "soft" acid. This results in platinum having a greater affinity for sulfur and nitrogen ligands. This affinity greatly determines the main biological targets for platinum(II) compounds, such as the sulfur of cysteine or methionine and nitrogen of guanine or adenine. The cysteine and methionine represent the protein adduct of binding, while the guanine and adenine represent the DNA adduct. Cisplatin and similar compounds possess a high specificity when binding so reactions tend to progress slowly.

Cisplatin takes a specific path in the body and further within the cells due to its geometry and *cis* conformation. Cisplatin is distributed intravenously where it remains neutral in the high chloride (Cl^-) concentration environment. The neutrality of the platinum compound allows it to pass through the cell membrane into the cytosol of the cell, which contains a lower Cl^- concentration. The lower Cl^- concentration causes the chlorides to be displaced by water molecules. This substitution charges the overall compound preventing it from easily leaving the cell. This new cisplatin-water compound can react with DNA adducts, protein adducts, or other adducts in the cell. Reactions with protein adducts are thought to lead to detoxification, toxicity, or transfer to DNA. Reactions with DNA adducts lead to either cell repair or cell death.

Cisplatin's effectiveness in the body in preventing tumor growth comes by distorting the DNA within the cell. The main site of reaction with DNA is at the N7 atom of guanine, and the major adduct that forms is a 1,2-intrastrand cross-link between adjacent guanines. The cisplatin-DNA cross-link formation distorts the DNA structurally preventing it from replicating. The distortion of the DNA is the main mechanism in its antitumor activity by preventing cancer cells from replicating into new tumor cells. The *trans* isomer is not able to form the 1,2-intrastrand cross-link, but leads to a different distortion, which is one of the suspected reasons that this isomer is less effective at anticancer activity. This binding of cisplatin to DNA for distortion is controlled kinetically and leads to cytotoxicity.[3] Cisplatin is known to be toxic, carcinogenic, teratogenic, and mutagenic, as well as to have interactions with several other kinds of substances. Side effects of the drug during treatments can include renal toxicity, nephrotoxicity, hypomagnesaemia, nausea, vomiting, diarrhea, ototoxicity, neurotoxicity, hematological effects, sensitivity reactions, cardiovascular effects, ocular effects, and hepatic effects.[5] It is apparent that there is targeted cell death throughout the entire body and not just where cancer or a specific tumor is present. Due to its extreme side effects, efforts are being made to alter the structure or functionality of the platinum compound to reduce the amount of damage done to non-cancerous cells in the body.

Methionine is an amino acid that contains a side chain with a thioether group[6]. As stated earlier, Sherman and Lippard[3] noted that the type of platinum(II) form in cisplatin has a high affinity for sulfur, which makes methionine a prime target for reaction. Although, the platinum would have a high affinity for sulfur, it is also capable of coordinating to a nitrogen or oxygen in the compound. As mentioned earlier, platinum(II) is a soft acid and its reactivity can be related according to the hard-soft acid-base theory. Assignment of a hard or soft characteristic is relative to one element being compared to another, such as one atom being harder or softer when compared to another atom. This theory can be applied to two atoms of interest in reaction to

cisplatin: sulfur and selenium. Elements are considered softer if they have a larger radius, lower electronegativity, and higher polarizability, which can represent movement down a group on the periodic table among nonmetals. Selenium is one below sulfur in the Group VI elements. The theory states that soft acids will prefer to react with soft bases, and hard acids will prefer to react with hard bases. Since selenium is a softer element that sulfur, it is likely that it will react with the soft platinum(II).[7]

Selenomethionine is a natural amino acid that has the same structure as methionine with a selenium atom substituted for the sulfur atom[6]. Since selenium is considered to be softer than sulfur it can be predicted that cisplatin and similar compounds would react with selenomethionine than methionine. Researcher Steve Chmley has previously done experiments using an analog of cisplatin ([Pt(dien)Cl]Cl) and reacting it with both methionine and selenomethionine. The results that he gathered suggested that the reaction between [Pt(dien)Cl]Cl and selenomethionine was kinetically favored over its reaction with methionine. The experiments also showed that over time an equilibrium between the product of [Pt(dien)Cl]Cl with methionine and the product with selenomethionine was established.

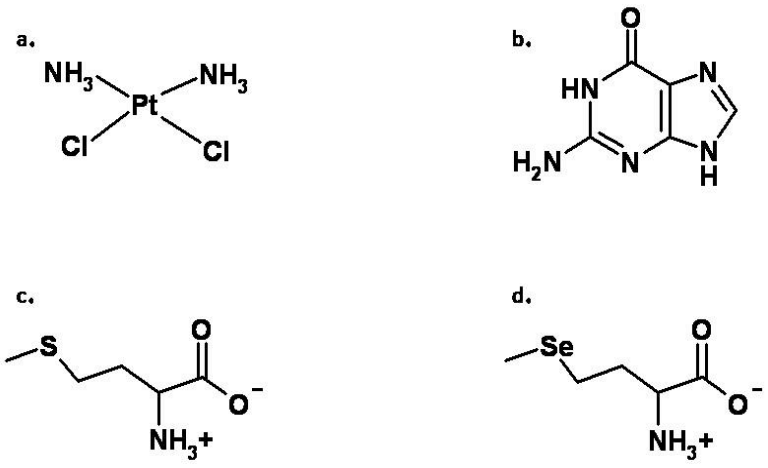

Figure 1: Structurees of a. cisplatin; b. guanine; c. methionine (Met); d. selenomethionine (SeMet).

Previous experiments have shown that cisplatin is capable of binding to the DNA adduct in the cell and distorting the DNA to prevent replication. The platinum(II) form that makes up cisplatin is a soft metal that has been shown to react faster with softer selenomethionine, but comes to an equilibrium with methionine and selenomethionine. This project was about reacting selenomethionine with two analogs of cisplatin: $Pt(Me_4en)(NO_3)_2$ and $Pt(en)(NO_3)_2$. In previous experiments with a $Pt(Me_4en)^{2+}$ compound, reactions with Met and N-AcMet have resulted in the formation of one product. It is thought to be due to the steric hindrance of the platinum compound's structure (as shown in Figure 2). Reaction of Met and N-AcMet with a $Pt(en)^{2+}$ compound is able to form multiple products due to a lack of steric hindrance and multiple substitution positions available.

Figure 2: Structures of a. $Pt(Me_4en)(NO_3)_2$ and b. $Pt(en)(NO_3)_2$.

Two main techniques were used in the collection of data about the structures and properties of the compounds and their products: Nuclear Magnetic Resonance Spectroscopy and High-performance liquid chromatography. Nuclear Magnetic Resonance (NMR) is a phenomenon in which the nuclei of particular atoms are immersed in a static magnetic field and exposed to a second oscillating magnetic field. Spectroscopy is the phenomenon of the interaction of matter with electromagnetic radiation. NMR spectroscopy uses the NMR phenomenon to study the chemical, physical, and biological properties of matter. Many atomic nuclei, such as hydrogen (^1H) and platinum (^{195}Pt), can be imagined as spinning around an axis, said to be a nuclear spin. The charge of the molecule causes its nuclear spin to create a tiny

magnetic field, which can then be manipulated by a larger magnetic field (or external magnetic field).

In a simple case, when exposed to the external magnetic field, the nuclear spin can have one of two orientations: aligned (α spin state) or against (β spin state). The aligned orientation is energetically favorable, while the against is higher in energy. The right frequency produces resonance when irradiation bridges the difference in energy between the two states by "flipping" from one state to another. Afterward, it returns to its original state, and continues a cycle of constant excitation and relaxation of the nuclei. The difference in the energy between the spin states is directly related to the external field strength, which is directly proportional to the absorption frequency. The energy of this absorption is referred to as the chemical shift. The chemical shift of nuclei depends on the electron density surrounding it. Therefore, the chemical shifts of nuclei can be used to determine the molecular structure of a species by the variations in the electron densities around them.[8]

Another technique being used is high-performance liquid chromatography. High-performance liquid chromatography (HPLC) is an enhanced version of column chromatography used to identify, separate, and quantify compounds. HPLC makes use of a column that contains a "packing material" called the stationary phase, a pumping system that pushes the mobile phase through the column, and a detector. The stationary phase is part of the column and is what the solutes in the mobile phase travel through and with which they interact. The mobile phase is the part containing the solutes that interact with the stationary phase through the column. This kind of technique takes advantage of a high affinity of proteins for specific chemical groups. This project used cation exchange chromatography that is based on the separation of polar molecules and ions due to the charge properties of the species. In cation exchange chromatography, positively charged molecules are attracted to the negatively charged stationary phase.[9]

These techniques were used in the project to characterize platinum (II) compounds containing two leaving groups. The techniques were used to determine key reaction characteristics in the two analogs of cisplatin being investigated in the project, and structural characteristics of the product(s) formed after reaction with selenomethionine.

MATERIALS AND METHODS

Synthesis of Pt compounds. $Pt(en)Cl_2$ and $Pt(Me_4en)Cl_2$ were synthesized based on a modification[10] of a previous method of Romeo et al[11]. The chloride compounds were converted to nitrate (NO^-_3) by addition of two equivalents of $AgNO_3$, stirring in dark for 24 hours, filtering, and drying.

Reaction of Pt compounds with L-SeMet. One or two molar equivalents of L-SeMet were reacted with $Pt(Me_4en)(NO_3)_2$ and the pH was adjusted to ~5.0. $Pt(en)(NO_3)_2$ was reacted with L-SeMet and L-Met in 1:1, 2:1, or 1:3 Pt:ligand ratios and adjusted to a pH of ~5.0.

NMR Spectroscopy. Both ^1H and ^{195}Pt NMR spectra were attained on a JOEL Eclipse 500 MHz NMR instrument. The ^1H NMR spectra obtained were adjusted for temperature and referenced using the residual HOD signal relative to TSP. ^{195}Pt NMR spectra were referenced relative to K_2PtCl_6 (0 ppm).

HPLC (High-performance liquid chromatography). The project used cation exchange chromatography column to separate the three products of the reaction of $Pt(en)(NO_3)_2$ with L-SeMet. The sample was eluded with 20 mM phosphate, pH 6 (Buffer A) and, 20 mM phosphate, pH 6.0 and 0.5 M NaCl (Buffer B). Time zero minutes was a flow of 100% Buffer A, at 20 minutes there was 50:50 mixture of Buffer A and B, and after 25 minutes it returned to 100% of Buffer A. The flow rate through the column was regulated at 0.5 milliliters/minute. The sample was not collected after separation.

RESULTS

Reaction of $Pt(Me_4en)(NO_3)_2$ with SeMet. The addition of one equivalent of SeMet to $[Pt(Me_4en)(H_2O)_2]^{2+}$ showed new resonances with singlets at 2.49 and 2.51 ppm in the ^1H NMR spectrum. Previous reaction between $[Pt(Me_4en)(H_2O)_2]^{2+}$ and Met led to similar sets of resonances[12]; the product was assigned to $[Pt(Me_4en)(Met\text{-}S,N)]^+$, and the two singlets are due to slow chirality exchange at the S atom. Thus, the signals in the SeMet spectrum are assigned to a single product, $[Pt(Me_4en)(SeMet\text{-}Se,N)]^+$, with differing chirality around the selenium atom. There was a 1:1 ratio between $Pt(Me_4en)^{2+}$ and SeMet based on integration of the NMR signals. Support for the assignment came from the ^{195}Pt NMR spectrum, which showed two peaks at -3190 and -3260 ppm. These results were similar to the values of -3160 and -3230 ppm for Met and $Pt(Me_4en)^{2+}$, and to the product of $[Pt(dien)(SeMet\text{-}Se)]^+$.

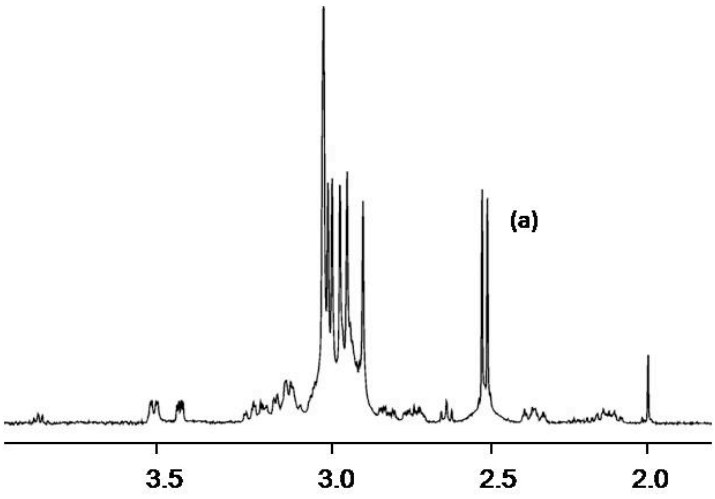

Figure 3: Reaction of $Pt(Me_4en)(NO_3)_2$ [Me_4en = N,N,N',N'-tetramethylethylenediamine] with SeMet yields one product, $[Pt(Me_4en)(SeMet\text{-}Se,N)]^{+1}$ (a). The two peaks correspond to a slow exchange of chiralities around the Se atom. The *trans* effect is much slower in this reaction.

Reaction of $Pt(en)(NO_3)_2$ with SeMet. The addition of SeMet to $Pt(en)(NO_3)_2$ showed multiple peaks ranging from 2.4 to 2.5 ppm leading to three different products: monochelate, bis, and bischelate. The monochelate product showed resonances with a set of two singlets at 2.4

ppm in the NMR spectrum. The resonances were dominant in reactions containing low SeMet:Pt ratios. Reaction of $Pt(Me_4en)(NO_3)_2$ with SeMet led to similar resonance signals. The bis product showed resonances with two singlets at 2.5 ppm in the NMR spectrum. These resonances were dominant in reactions containing higher SeMet:Pt ratios. The en signal at 2.7 ppm is a singlet relating to its C_2 symmetry. The bischelate product showed resonances with one singlet and two doublets between 2.4-2.5 ppm in the NMR spectrum. There is also the presence of free en indicated by a signal at 3.35 ppm. This product is formed from the bis product with the presence of excess SeMet. The doublet at 2.5 ppm related to the bis product disappears as the bischelate singlet and doublets between 2.4-2.5 ppm appears. This product does not form from the monochelate product even in the presence of excess SeMet.

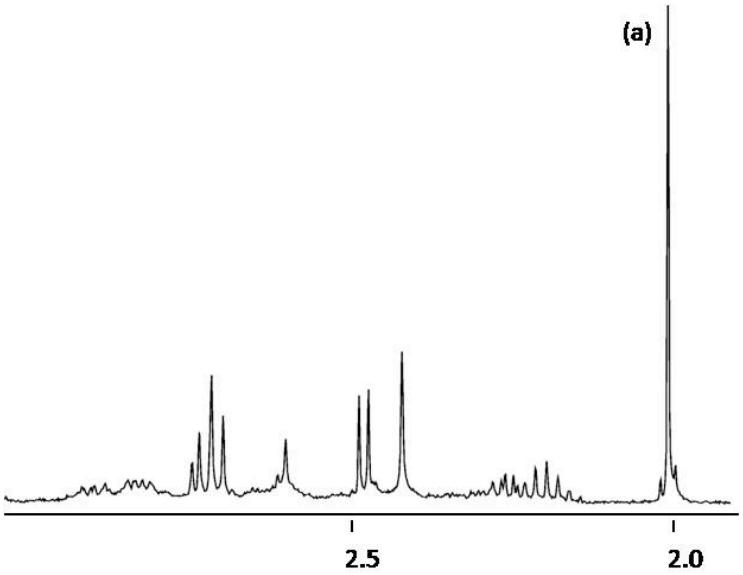

Figure 4: The reaction of $Pt(en)(NO_3)_2$ with an excess of SeMet (a) results in the formation of three separate products: bis, bis chelate, and monochelate. The sample is shown at a pH of 7 about one hour after the Pt(en) and SeMet solutions were mixed.

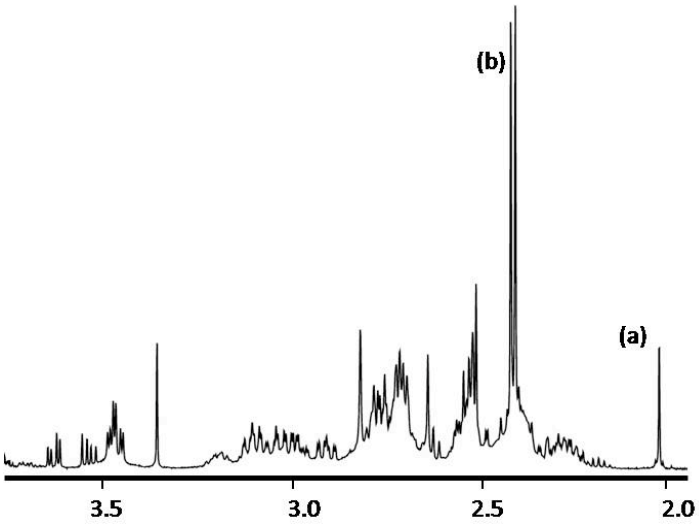

Figure 5a: The monochelate product (b) is favored when $Pt(en)(NO_3)_2$ is in excess and the SeMet (a) is added to the sample in timed increments while maintained at a pH of around 5.0.

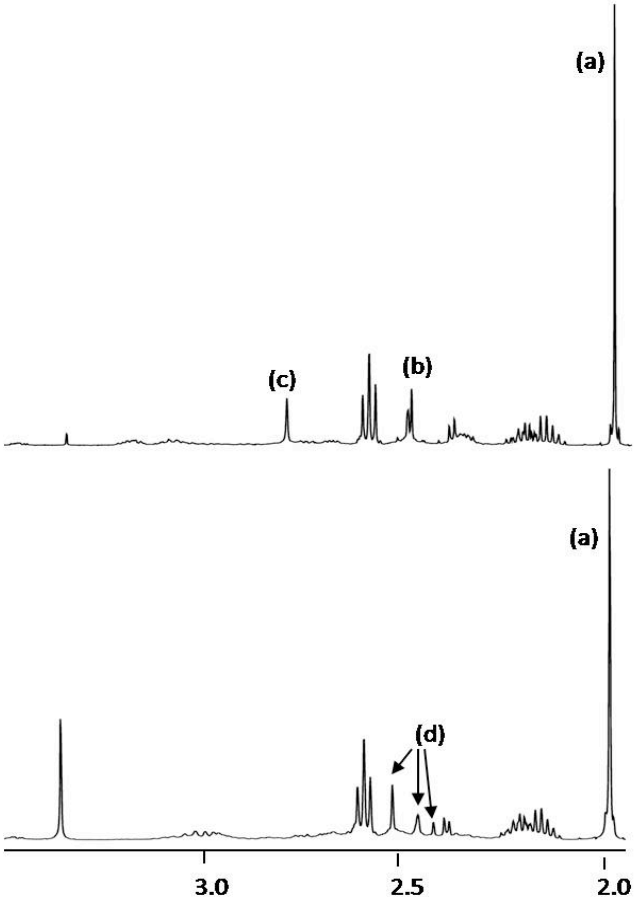

Figure 5b: (a) Selenomethionine, (b) bis product, (c) en signal corresponding to the bis product, and (d) bis chelate product taken 20-30 minutes after mixing at a pH of about 5.0 and again 2 days later at pH about 5.0.

This reaction was also analyzed with high-performance liquid chromatography (HPLC). The three products from the reaction of $Pt(en)(NO_3)_2$ with SeMet were separated by their different charges using a Hitachi Elite LaChrom: Pump L-2130 and UV Detector L-2400 cation exchange column. The bischelate product is neutral and was the fastest product to travel through the column indicated by a peak at 2-3 minutes. The monochelate product has a charge of +1 associated with it and was indicated by a peak at 5 minutes. The bis product has a charge of +2 associated with it and was indicated by a peak at 15 minutes to move through the column. The buffers used to move the sample through the column were 20 mM phosphate, pH 6 and 0.5 M NaCl.

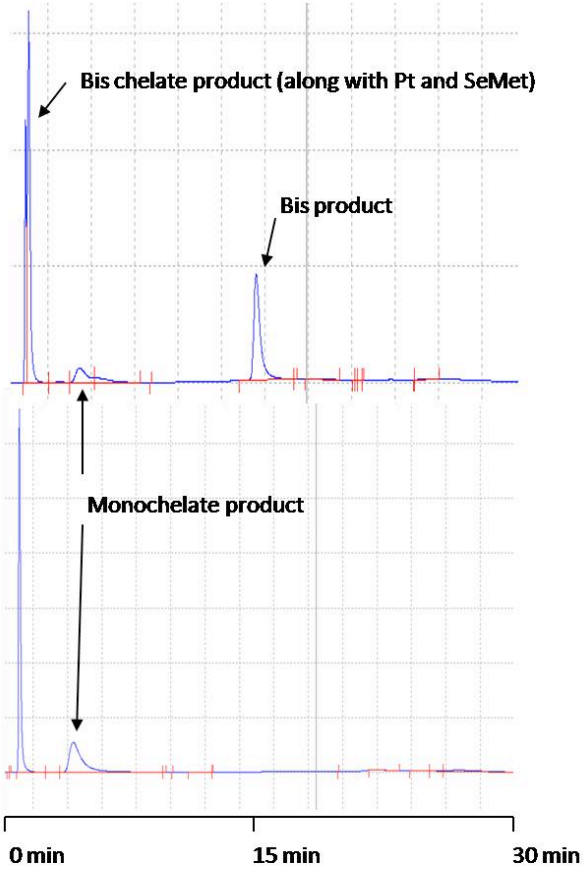

Figure 6: HPLC data with a cation exchange column for the three products of bis, bis chelate, and monochelate. The bis chelate product has a neutral charge and is ejected initially along with Pt(en) and SeMet, while the monochelate product (with a +1 charge) ejects at ~4.5 min. and bis product (with a +2 charge) ejects at ~15 min.

DISCUSSION

It has been shown in previous experiments that the type of platinum(II) form making up the structure of cisplatin reacts faster kinetically with SeMet than Met. Expanding on this suggestion, it was the purpose of this project to take two analogs of the cisplatin compound and react them with SeMet to characterize their products. Both the analogs have two leaving groups of either chlorides or nitrates that relate to the structure of cisplatin. The two leaving groups are important for creating an overall chemistry to easily move the compound through the cell membrane and then be replaced to prevent it leaving the cell. The differences in the analogs from cisplatin were meant to look at the effects that bulk can play in binding with SeMet.

The results of the project in certain areas can be used to formulate a prediction of the SeMet adduct in these reactions. It is possible to predict the SeMet adduct characterization with the behavior of the reaction of $Pt(Me_4en)$ with SeMet. The product $[Pt(Me_4en)(SeMet)\text{-}Se,N]^+$ with its two chiralites were represented by specific peaks in both the ^1H and ^{195}Pt NMR spectra, which were similar to peaks seen previously with the reaction of $Pt(Me_4en)$ with N-actylmethionine (N-AcMet) and Met. This allows for certain similarities to be drawn regarding the structure and other characteristics of the product.

One of the main focuses of the results with the project has been on a phenomenon called the trans effect. The trans effect is related to platinum(II) compounds and suggests that ligands trans to a chloride, sulfur, or selenium atom are more easily displaced than when trans to an ammonia group. This effect is known to be a kinetic effect and not thermodynamic, meaning that it requires a compound effective at substituting for the reaction to work properly. It prefers to interact with ligands or elements that are kinetically favored, as opposed to those that are thermodynamically favored.[7] There are several culminating factors that suggest this effect is taking place in some of the reactions and not taking place in others.

The reaction of SeMet with Pt(en) results in separate products dependent on the Pt:Se ratio present. In a low Se:Pt ratio the monochelate product is prominent, while in a high ratio the bis and bischelate product is more prominent. The low Se:Pt ratio favors the monochelate product mainly due to there being less SeMet compounds to Pt compounds, making the probability of double substitution with SeMet unlikely. Although the substitution with SeMet is faster than the formation of the chelate on the monochelate product, without a second substitution (due to low concentration) the nitrogen of one of the amine groups will chelate and prevent further substitution. The bischelate product formed from the reaction of Pt(en) with SeMet in a high Se:Pt ratio only appears after the formation of the bis product. In ^{1}H NMR spectra taken at several intervals over a period of time, the signals for the bis product disappear and the bischelate signals begin to form. There are no peaks indicating that the bischelate product is present at the initial stage of the reaction. In a reaction with low Se:Pt ratio favoring the monochelate product, there appears no formation of the bischelate product. This is due to the presence of the monochelate labilizing only one of the Pt-N bonds, which directly affects the formation of the bischelate product by breaking the chelate. Formation of a new chelate would be unfavoarable, therefore there would be no formation of a bischelate product. Even adding excess of N-AcMet after forming a monochelate product with N-AcMet, it took approximately three months at a pH of ~2.0 before any change was observed.

The process of the trans effect with this compound is achieved by the chelated ligands that are displaced when the sulfur or selenium ligand attaches trans to both nitrogen ligands. The entire process uses the formation of two chelates to replace one. The first step in the process is to replace an N,N chelate (two chelated amine groups) with an Se,N chelate. The first step is assumed to be thermodynamically neutral. The second step is to displace the rest of the N,N chelate to form a second Se,N chelate, which should be favorable. The free N,N chelate (free en)

is then visible with its own signal. At no point was there indication that an intermediate form was being observed in the NMR data.

Many studies have been done with cis/carbo-platin in which the amine groups are not chelated. In those experiments that were performed, the results suggested that the non-chelated amine groups were easier to displace than chelated amines. This is strongly influenced by the chelate forming a more rigid structure to the central atom.[13]

Platinum (II) has a high affinity for sulfur, and it is possible that sulfur-containing biomolecules other than DNA may have an important role in the metabolism and mechanism of action or cisplatin. Higher affinity for selenium may cause it to have the same role as sulfur, and it has been shown in *in vivo* studies to prevent cisplatin-induced drug resistance and toxicity. However, it does not affect the cytotoxicity of cisplatin. Greater affinity for SeMet, along with cisplatin's high specificity, may lead to new methods of targeting and reactions within the cell.[14]

Although the specific mechanisms involved in the reaction of two cisplatin-like compounds with selenomethionine are not fully understood, the *trans* effect plays a significant role in one of them. The reactions produced four products that were identified and characterized from NMR spectra and HPLC data and the significance of these products could be the focus of future research. The anticancer properties of selenomethionine and its reduced cellular toxicity make it a promising area for further cancer reaserch. The knowledge gained from the project could provide insight into the specific mechanisms that occur in the reactions and if they will have significance in reactions with other molecules.

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