An Investigation into the Essential Residues in the Interaction Between Cisplatin and the C-Terminus of hCTR1

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Abstract: Cisplatin has been used as an anti cancer agent for many years. However, the exact mechanism for the cellular uptake of cisplatin is still not completely understood. Research into the involvement of the protein Human Copper Transporter 1 in the uptake process was performed, focusing in particular on the carboxy-terminus to determine the essential residues in the interaction with cisplatin. Ultraviolet spectroscopy, two-dimensional heteronuclear single quantum coherence nuclear magnetic resonance spectroscopy, and electrospray ionization mass spectrometry were all utilized in the investigation. The major binding sites were found to be the cysteine residue and terminal ammine of the peptide, with the peptide being singly platinated at low cisplatin concentrations and bis-platinated or tris-platinated at high cisplatin concentrations. These results suggest that the cisplatin initially binds to the cysteine residue and is then transferred within the peptide to the terminal ammine binding site to allow for further platination. With an improved understanding of cisplatin's interaction with Human Copper Transporter 1, more methods of overcoming the drug delivery obstacle can be introduced.

Introduction: Cisplatin is a compound that has been used in chemotherapy in order to combat cancer for many years. Cispaltin is a square planar compound containing two *cis* chloride ligands and two *cis* ammine ligands. The general reaction of a square planar compound can be described as associative substitution, in which both the incoming ligand and the outgoing ligand influence the reaction. During the substitution reaction, the incoming nucleophile can attack from either the top or the bottom of the plane. As the nucleophile binds to the central atom, the complex

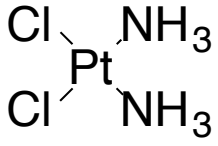

Figure 1. Cisplatin Structure The square planar structure is shown, containing ammine ligands in the *cis* position and two chloride ligands in the *cis* position.

coordinates to form a trigonal bipyramidal intermediate before the leaving group departs.

A major obstacle in the use of cisplatin to fight cancer is the delivery method. Various techniques have been used including enclosing cisplatin in a variety of nanoparticles.[1, 2] However, the exact mechanism in which the complex travels into cells is still unclear. Learning more about how cisplatin and other platinum complexes enter cells could potentially lead to an increase in the effectiveness of cancer treatment.

The prevailing theory is that cisplatin passively diffuses into cells and then undergoes aquation.[3] Aquation is a substitution reaction in which a ligand is replaced by water. In the case of cisplatin, the water replaces one of the chloride ligands. Recently, the involvement of a specific copper transporter protein, CTR1, has been investigated in the process of the cellular uptake of cisplatin. Research conducted by Nitiss has shown that the removal or mutations of the genes which express CTR1 leads to a large increase in cisplatin resistance.[4] These results suggest that CTR1 is somehow significant to the uptake process of cisplatin.

The human form of CTR1 (hCTR1) is composed of 190 amino acid residues. Furthermore, hCTR1 exists as trimers. A trimer is formed when three peptides, or monomers, operate together. Each monomer contains one transmembrane structure, one amino-terminus that exists outside of the cell, and one intracellular carboxy-terminus.[5] It has been shown that

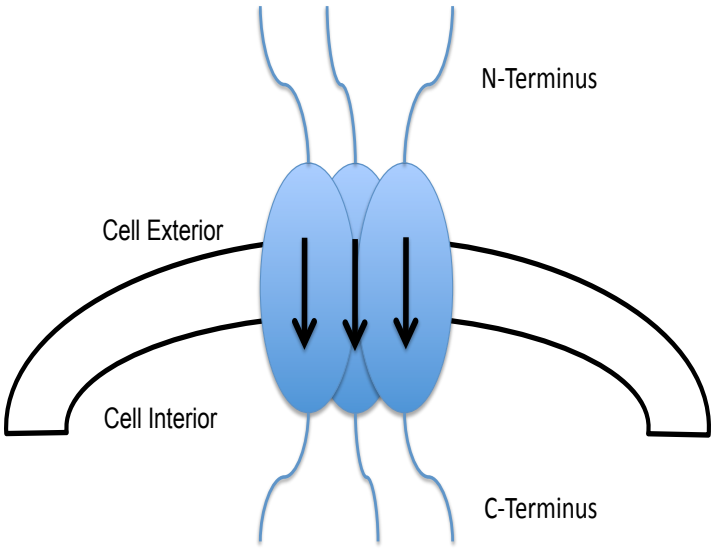

Figure 2. Structure of hCTR1 Trimer Conformation of three hCTR1 monomers with the N-Terminus outside the cell and C-Terminus inside the cell. Adapted from Wang et al.

removing the C-terminus from hCTR1 removes the protein's ability to uptake cisplatin.[6] This behavior indicates that the C-terminal portion of the protein is vital to the uptake of cisplatin and similar platinum based anticancer compounds.

In particular, the C-terminus corresponds to the 183-190 residues of hCTR1. This octapeptide contains the following residue sequence: valine, aspartic acid, isoleucine, threonine, glutamic acid, histidine, cysteine, and histidine (Figure 3). In order to investigate the interaction between cisplatin and the C-terminus, Wang et al. utilized a model C8 peptide that consisted of the same residue sequence. Multiple experiments were conducted to determine the essential residue or residues and their interactions with cisplatin. These experiments used ultraviolet (UV) spectroscopy, 2D ^{1}H-^{15}N Heteronuclear Single Quantum Coherence Nucelar Magnetic Resonance (2D ^{1}H-^{15}N HSQC NMR), and electrospray ionization mass spectrometry.

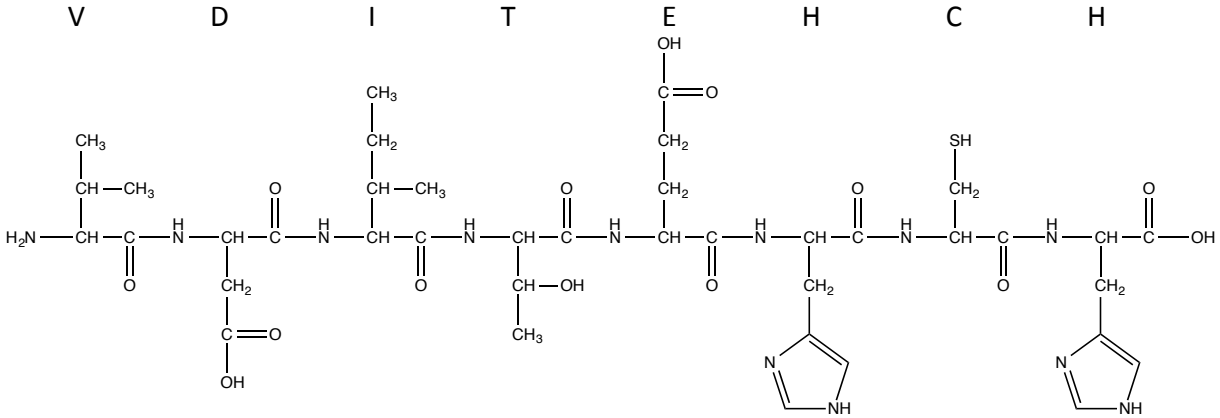

Figure 3. Composition of C8 Peptide Labeled residues of the VDITEHCH model peptide.

The first technique used by the researchers was UV spectroscopy. Previous research had suggested that cysteine residues react quickly with platinum. One reason for this reactivity is that cysteine is considered a thiolate when deprotonated, thiolates are classified as soft acids. Furthermore, platinum is classified as a soft base. Soft acids tend to have low charge densities, meaning they are larger and possess weaker charges (1+, 2+). On the other hand, hard acids

have higher charge densities, are strongly charged and are relatively small molecules. The same pattern occurs with bases. Soft acids generally react with soft bases while hard acids react with hard bases. The experiment was meant to investigate if the same type of reaction occurred in this specific interaction.

Ultraviolet-visible spectroscopy is a technique that can provide information regarding the identity, absorbance, and concentration of a substance. UV spectroscopy is based around the sample's ability to absorb ultraviolet light. The UV spectrum ranges from 100nm to 400nm. Molecules in the sample are irradiated with ultraviolet light, which has a fairly large amount of energy. The electrons in the molecule or compound react to this energy in different manners depending on which type of molecular orbital the electrons occupy. Electrons located in sigma orbitals are the most stable because they exhibit the most overlap between the bonding orbitals. Sigma orbitals therefore require the largest amount of energy to become excited and change levels. Because these electrons need larger amounts of energy to become excited they would absorb light at higher energy levels and lower wavelengths. Compounds that contain double and triple bonds have electrons that occupy pi orbitals. Electrons in pi orbitals are less stable, due to their higher energy level, than electrons located in sigma orbitals. As a result, electrons in pi orbitals absorb light at lower energy levels and higher wavelengths. Finally, unbonded electrons, or lone pairs, have higher energy levels than electrons in both sigma and pi orbitals and are the least stable. The instability of these electrons means that an even smaller amount of energy is needed to excite them and cause a shift in energy levels. This small amount of energy correlates to a large wavelength for lone pairs and unbonded electrons.[7] Because the researchers were looking for the specific interaction between sulfur and platinum they were able to focus on the precise wavelength that the reaction corresponds to and monitor absorbance values.

In order to investigate how the cysteine residue interacts with cisplatin, 2D ^{1}H-^{15}N HSQC NMR was used. NMR is a technique that is often used to determine the structure of molecules. Nuclei in molecules are always spinning, which generates a magnetic field. In NMR experiments, the nuclei are placed in magnetic fields and are irradiated with radio frequency (RF) energy. When protons in the nuclei are hit with the RF energy they move to one of two spin states. There is a slight difference in the energy levels between the two available spin states. When the incoming RF energy equals this difference in energy between the energy levels the protons switch spin states, creating a signal that the NMR can read and record. Different electronic environments affect the signal, or chemical shift, of nuclei. Protons in electron rich environments are shielded more from the incoming RF energy, creating a smaller energy difference between the two spin states. The smaller energy difference means that a lower frequency is required to make the protons switch states; this lower frequency corresponds to a lower chemical shift. The opposite trend is seen in electron poor environments, the proton is less shielded from the field and experiences a higher difference in energy levels. Higher frequencies are required to make the protons switch states, corresponding to higher chemical shifts.[8]

HSQC NMR is based around the idea of using two different NMR spectra in conjunction with each other to gain more information. The two spectra correspond to different nuclei. In this experiment the spectra correspond to ^{1}H and ^{15}N nuclei. These two spectra represent the axes of the two-dimensional spectrum. The spectra operate in the same manner as they would independently of a two-dimensional spectrum. The two-dimensional spectrum is used to show interactions between the two one-dimensional spectra. Peaks in the HSQC spectrum represent instances in which the two different nuclei have coupled spin states; generally this interaction occurs when the nuclei are separated by one bond. Each peak in the HSQC spectrum

correlates to one peak in the ^{1}H spectrum and one peak in the ^{15}N spectrum. The use of this NMR technique provided detail into how the cysteine residue, and nearby residues, interacted with cisplatin.

The final technique used was electrospray ionization mass spectrometry. This technique was used in order to gain more insight into what extent the model peptide was platinated, meaning a platinum compound bound to the peptide. ESI MS is often used to study large biological macromolecules. First, the molecules in solution are ionized by being sprayed through an electrically charged aerosol apparatus. This process results in many small droplets of charged molecules. However, in order to proceed, the sample needs to be in the gaseous state. The solvent is then evaporated through increasing temperatures in the ESI chamber. As the solvent evaporates and the charge droplets decrease in size the ions escape into the gaseous state. Once the ions have been transferred from solution into gas they are entered into the quadrupole mass analyzer.

The mass analyzer consists of four metal poles that are parallel to each other. These poles are arranged in equal distances from each other in a square. Each pole is electrically charged in tandem with the pole across from it. The ions pass through the chamber in parallel to the poles. As the ions travel through the chamber the two sets of poles alternate charges. This rapid change of charges causes the ions to oscillate up and down while travelling through the chamber. Each unique ion has its

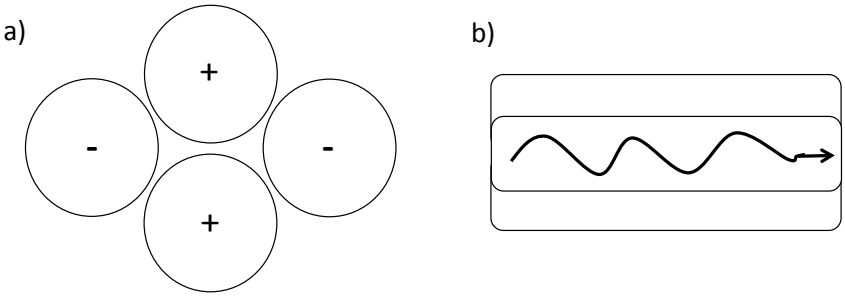

Figure 4. Mass Spectrometry Quadrupole Diagram showing a) the top down view of the quadupole and potential charge arrangement and b) a possible path an ion can take as it travels parallel to the poles.

own mass to charge ratio (m/z), which results in distinct movement patterns for different ions. A detector at the end of the chamber monitors the movements of the ions and calculates the mass to charge ratio based on the speed at which the poles change charges. Once an experimental m/z value is obtained it can be compared with calculated values of possible products.

Tandem mass spectrometry is a technique very similar to that described above. However, instead of one quadrupole chamber there are three. The poles in the first chamber are programmed to allow only one desired ion to reach the detector. The mass to charge ratios of the other ions cause them to oscillate too much which results in collisions with the poles and an inability to reach the detector. Once the desired ion reaches the second chamber it is further broken down and ionized by colliding with an inert gas. These ions enter the third chamber, which is similar to the first, and are separated by their unique mass to charge ratios.[9] Tandem mass spectrometry allows for the further fragmentation of samples, which can give more insight into the structure of the sample. Using mass spectrometry allows for information to be gathered regarding the extent of platination of the peptide during the interaction.

With the use of UV spectroscopy, 2D ^{1}H-^{15}N HSQC NMR, and ESI MS/MS data it was possible to determine the essential residue of the C-terminus of hCTR1 and investigate its behavior in the interaction with cisplatin.

Results and Discussion *UV Spectroscopy of C8 Peptide and Cisplatin Interaction:*

Previous research has shown that cysteine residues tend to bind to platinum complexes. The R group of cysteine at neutral pH levels consists of one carbon atom bonded to a protonated sulfur atom. Such an interaction would create a single bond between the sulfur of the cysteine residue and the platinum atom itself. To maintain a four membered complex a chloride ligand would most likely serve as the leaving group due to its status as a weak base

(Figure 5). In order to determine if the cysteine residue of the C8 terminus binds with platinum complexes, UV spectroscopy was employed. Over a span of 14 hours, 0.5 mM cisplatin was reacted with 0.5 mM C8 peptide. The results showed a large spike in absorbance at a wavelength of approximately 280nm. Ishikawa has shown that bonds between sulfur and platinum absorb light at 280nm.[10] A wavelength of 280nm is relatively low, suggesting that there are no electrons in pi orbitals present. With no electrons in pi orbitals the interaction must be a single bond. The UV spectroscopy results indicate that a single bond forms between the sulfur atom of the cysteine residue and the platinum atom when the C8 peptide is reacted with cisplatin. These results coincide with the possible associative mechanism proposed earlier.

This experiment suggests an important point about the reaction between the C8 peptide and cisplatin. There is an interaction between the cysteine residue and the platinum atom of cisplatin. Knowing that the platinum in cisplatin bonds directly to the cysteine residue it is important to investigate further how the inorganic complex reacts with the residue and the remainder of the peptide.

2D NMR of C8 Peptide and Cisplatin interaction: In order to observe the interaction on a more detailed level, 2D ^{1}H-^{15}N HSQC NMR was performed. Samples from the reaction were run after 1.5, 3, 8, and 12 hours. HSQC NMR is a versatile technique that allows for simplified identification of unknowns and the observation of reaction mechanisms as reactions progress. In this experiment ^{15}N-labeled cisplatin was reacted with the C8 peptide. Using HSQC in this case illustrates the interactions between the hydrogen and nitrogen atoms in the reaction. More specifically, the experiment provides information about the ammine ligands of cisplatin throughout the reaction due to the fact that the only ^{15}Nitrogen atoms present in the reaction should be in the ammine ligands of cisplatin. This fact means that the conformation of the cisplatin and peptide complex can be further evaluated.

There are numerous strong, well defined cross peaks at around -68 (^{15}N) and 4.1 (^{1}H) ppm after the reaction has proceeded for 1.5 hours (Figure 6). These peaks are in a region that corresponds to the ammine ligand that is *trans* to the chlorine ligand. These peaks make sense because both the ammine ligands of cisplatin are *trans* to chlorine ligands. As the reaction progressed the intensity and number of cross peaks in this region steadily declined. There is a significant difference between the number of cross peaks after 1.5 hours and the number of cross peaks after eight hours and 12 hours. This change confirms that the reaction between cisplatin and the peptide was proceeding. Furthermore, the data gives insight into how the reaction occurs. The decline over time of cross peaks related to the ammine group *trans* to the chlorine ligand suggests that the chlorine ligand serves as the leaving group. This finding makes sense, as halides are effective leaving groups for substitution reactions due to the fact that they are weak bases.

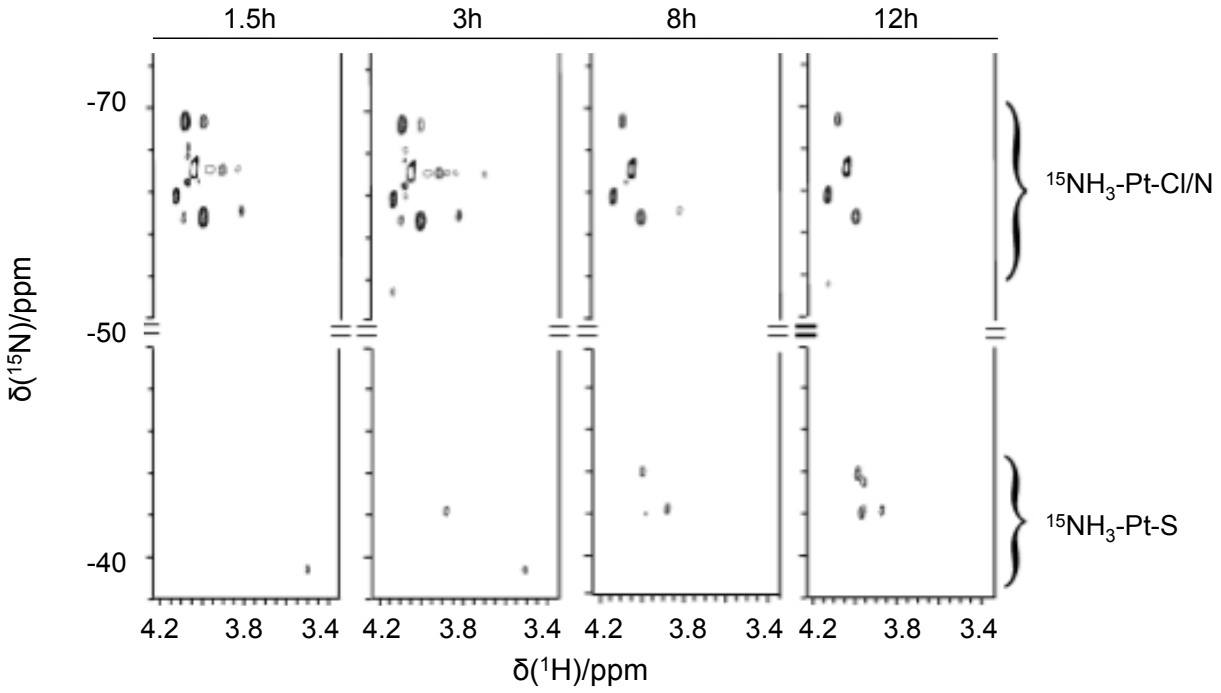

Figure 6. Cysteine Interaction With Cisplatin The 2D ^{1}H-^{15}N HSQC NMR monitored the reaction between 0.8 mM cisplatin and 0.8 mM C8 peptide in a 50 mM phosphate buffer with pH 7.0 at 25°C. The corresponding reaction times are given above each spectra. Adapted from Wang et al.

After the reaction proceeded for 1.5 hours there was a single, faint cross peak at -39 (^{15}N) and 3.5 (^{1}H) ppm (Figure 6). This cross peak is located in the region that corresponds to an ammine group *trans* to a sulfur atom.[11] As the reaction progressed the amount of cross peaks and their intensities increased. At eight hours and 12 hours the region looks significantly different than the region after only 1.5 hours. The indication that a bond forms between the platinum atom and a sulfur atom confirms that the cisplatin binds to the cysteine residue of the C8 peptide. Furthermore, the data provides a clearer picture of the mechanism for the interaction: the sulfur atom of the cysteine residue attacks the cisplatin and substitutes for one of the chlorine ligands, which serve as the leaving group. An important note regarding the experiment is that the reaction took place at a pH of 7.0. This pH is close to physiological pH, which means that the protonation states of the residues in the C8 peptide should be identical to the protonation states of the residues in the C-terminus during cisplatin delivery into the cell.

An interesting facet of the data presented in Figure 6 is the spectrum that was collected after 12 hours. There are still large amounts of cross peaks found in the region that correspond to the ammine ligand *trans* to the sulfur atom. This time span suggests that the platinum-sulfur bond is quite stable and that the ammine ligands are still present. However, previous research involving cisplatin interactions with glutathione has shown that as the platinum-sulfur bond is formed with the cysteine residue the platinum-ammine bond is broken instead of the platinum-chlorine bond.[12] One possible explanation for the difference observed in this experiment is the environment the interaction takes place. In the C8 peptide the cysteine residue is in the middle of two histidine residues. The sidechain of histidine consists of a carbon atom linked to an imidazole group. The imidazole group contains two nitrogen atoms that have the potential to form hydrogen bonds. As cisplatin binds to the cysteine residue it would make sense for the

complex to form hydrogen bonds with the nearby histidine residues for added stability. In order to form these hydrogen bonds the ammine ligands would have to be present because they are the only sites available to do so on cisplatin. Hydrogen bonds would provide additional stability within the complex so this idea serves as a potential explanation for the long-term existence of the adduct that is formed and for the continued presence of both ammine ligands on the compound.

A major factor in the interaction between cisplatin and the C8 peptide is the *trans* effect. The *trans* effect is a kinetic effect that influences substitution reactions. When positioned *trans* to the ligand that is leaving certain ligands increase the rate of the reaction. The two types of ligands that contribute to the *trans* effect are sigma donors and pi acceptors.

In the initial interaction between cisplatin and the C8 peptide both the chloride and ammine ligands of cisplatin are sigma donors because they both contribute one lone pair to the metal center. Sigma donors draw the electron density away from the metal center towards the bond with the *trans* ligand. This change in electron density weakens the bond between the metal and the leaving group, which facilitates a quicker substitution reaction. Chloride ligands are stronger sigma donors than ammine ligands because they are more electronegative. According to the *trans* effect one of the ammine ligands of cisplatin should leave in a substitution reaction. However, a chloride ligand leaves instead. This interesting difference can possibly be explained by the additional support provided by the hydrogen bonds discussed above. The added stability from the hydrogen bonds formed between the ammine and the histidine may outweigh the kinetic impact of the *trans* effect.

With the knowledge that the cysteine forms a stable single bond with the platinum atom of cisplatin that is possibly supported by nearby histidine residues further experimentation was performed in order to characterize the products of the reaction to a greater extent.

Mass Spectroscopy of Platination Products: Electrospray ionization mass spectroscopy was utilized to investigate the products formed from the interaction between cisplatin and the C8 peptide. Although the 2D HSQC NMR experiment provided information about which bonds were breaking and forming, mass spectroscopy can reveal more information regarding the complexation and platination of the product or products. Samples were taken over a six-hour span from the reaction, which was run with 0.5 mM C8 peptide and 1.5mM cisplatin. The excess of cisplatin was used to allow the peptide to become platinated multiple times, if possible.

After 30 minutes the only product detected was a bis-platinated product, [C8+2Pt(NH_3)$_2$Cl]. This result indicates that the cysteine residue binds to the cisplatin relatively quickly. Furthermore, the bis-platinated product indicated that the peptide reacts with two molecules of cisplatin. This information was not apparent from the NMR spectra. As the reaction progressed more products were observed. A variety of bis-platinated and tris-platinated complexes were detected. Dipeptides linked by platinum bonds were also found to be present. These findings show that although the initial bis-platinated product [C8+2Pt(NH_3)$_2$Cl] forms quickly there are further interactions, most likely within the peptide, that lead to more products. This experiment only showed that the peptide becomes bis-platinated. The results do not indicate where the other platination sites are,

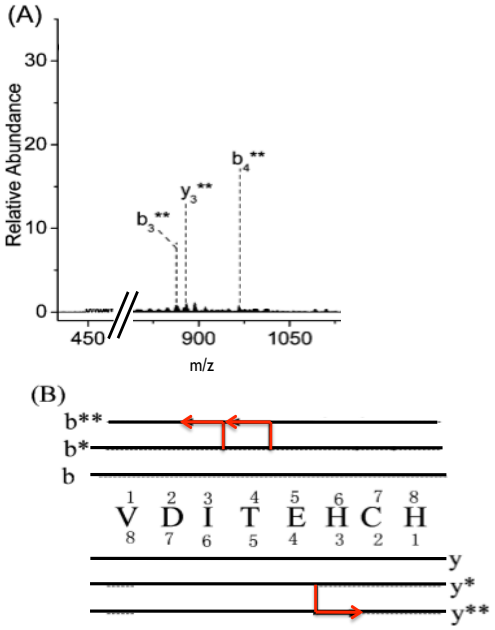

Figure 7. Platinated Peptide Fragments (A) Tandem mass spectrometry spectrum of [C8+Pt(NH_3)$_2$(PO_4)+Pt-(NH_3)$_2$]$^{3+}$. Bis-platinated fragments are denoted with a double asterisk. (B) Corresponding fragments displayed on C8. Bis-platinated fragments are represented by b** and y**. Adapted from Wang et al.

or how the further platination occurs.

In order to further investigate the binding sites of the C8 peptide and the further interactions suggested by the previous experiment a tandem MS/MS experiment was run. The precursor compound was $[C8+Pt(NH_3)_2(PO_4)+Pt(NH_3)_2]^{3+}$ (m/z= 502.36). This compound was obtained from an earlier ESI MS experiment, which was run in a phosphate buffer of pH 7.0 for 10 hours containing 0.5 mM cisplatin and equimolar C8 peptide. Similar to previous experiments, the pH level of 7.0 was intended to reflect how the C-terminus might be protonated. Fragments were identified based on calculated mass to charge ratios. All of the identified fragments in this experiment were determined to be bis-platinated, as shown in Figure 7. This makes sense since the fragments are smaller than what was tested in the initial ESI MS experiment. Smaller fragments mean fewer potential binding sites, making it unlikely that tris-platinated fragments would be found. One of the smallest fragments found was the y_3^{**} fragment, which corresponds to the HCH sequence in the C8 peptide. The fact that this fragment was found to be bis-platinated confirms that the HCH sequence plays an important role in the platination of the peptide.

Interestingly there were multiple bis-platinated fragments found that did not contain the HCH sequence. Both the b_3^{**}(VDI) and b_4^{**}(VDIT) fragments were bis-platinated and did not contain any part of the HCH sequence. The only difference between the two fragments is that the b_4^{**} has an extra residue, threonine. However, since both fragments were found to be bis-platinated it is reasonable to conclude that the threonine residue is not necessary for platination. That leaves the remaining parts of each fragment, the VDI sequence. According to the results of the tandem MS/MS experiment, the VDI sequence of the C8 peptide has the ability to undergo platination, as well as the HCH sequence. However, the NMR experiments did not generate data that suggested the VDI sequence underwent platination. A possible

explanation for this conflict is that the cysteine residue is the preferred binding position as the interaction starts and the cisplatin is then transferred to the VDI residue sequence through *trans* chelation. The hard-soft acid base theory supports this order of events. As stated before, the Pt^{2+} of cisplatin can be considered a soft acid due to its larger size and relatively low charge density. Likewise, the thiolate of cysteine can be viewed as a soft base for the same reasons. The only potential binding sites on the VDI sequence are the terminal ammine group and the carboxyl side chain of the aspartic acid residue. Due to their higher electronegativity, both of these sites would be considered harder bases than the thiolate, making them less favorable for the soft acid Pt^{2+}. Research has shown that platinum complexes migrate from sulfur containing residues to nitrogen containing residues in vivo.[13] One possible explanation for this phenomenon in this particular case is that by moving to the terminal ammine group the cisplatin molecule would reduce steric hindrance. It is likely that the platinum is transferred from the cysteine ligand to the terminal ammine group of the peptide.

Another ESI MS experiment similar to the initial setup discussed was run. However instead of running the reaction at a 3:1 cisplatin to C8 peptide ratio the experiment was run under equimolar conditions. This change was done to probe the initial uptake of the cisplatin. The equimolar conditions allow for less cisplatin to interact initially with the peptide. Although the experiment discussed above showed that initially the product was bis-platinated, that may have resulted from the high ratio of cisplatin. After the equimolar reaction proceeded for 0.5 hours two different mono-platinated products were detected. After the initial stage the abundance of these products decreased as bis-platinated and tris-platinated complexes were detected. These results confirm the idea that cisplatin initially binds to the cysteine residue and is then transferred to other residues on the peptide.

Although the primary purpose of hCTR1 is to transport copper complexes, the mechanism in which the transportation of copper occurs is quite different than the proposed mechanism for the transfer of cisplatin. The transportation of copper does not involve the C-terminus directly, unlike the cisplatin mechanism.[14] A possible reason for this difference is the disparity between the geometries of the two ions. While cisplatin is square planar, Cu^{2+} forms distorted octahedral compounds. Octahedral compounds operate under dissociative mechanisms, meaning that the leaving group has to leave entirely before the nucleophile can bond with the complex. The different geometry and reaction mechanism may explain why copper ions interact with different parts of hCTR1.

Conclusion: Through a series of experiments, the interaction between cisplatin and the C-terminus of hCTR1 has become clearer. Moreover, the preferred residue and its role have been identified. The UV spectroscopy data showed a large peak in absorbance at a wavelength of 280nm. This peak confirmed that the cisplatin was binding to the cysteine residue of the C8 peptide. The 2D HSQC ^{1}H-^{15}N NMR experiment provided more information regarding the bonding mechanism of cisplatin during the reaction. The data confirmed that platinum bonds directly to the sulfur of the cysteine residue. Furthermore, the data also indicated that the ammine ligands of cisplatin remain attached during the reaction. Finally, the compound was found to be quite stable, most likely as a result of hydrogen bonding opportunities provided by the ammine ligands. Mass spectrometry data suggested that initially the peptide only binds to a single molecule of cisplatin. However, after further reaction time the peptide undergoes further platination. Tandem mass spectrometry experimentation revealed that two sequences of residues were involved in the interaction with cisplatin: HCH and VDI. It

is likely that cisplatin initially binds with the cysteine residue and is then transported to the VDI sequence through *trans* chelation as another cisplatin molecule binds to the cysteine residue.

Overall, this was an ethical experiment. The tests were conducted on model peptides that were synthesized in a laboratory. Although cisplatin is used to treat cancer, the compound does have potential side effects. One serious side effect of cisplatin has been found to be kidney damage.[15] Due to the cisplatin and cisplatin derivatives that were generated as waste it is important that they were properly disposed of. Finally, the experiment has positive implications on the future. By learning more about how cisplatin, an anticancer agent, enters cells it is possible to further improve and fine tune cancer treatments involving platinum complexes.

Future experiments could focus more on the impacts of individual residues. The same C8 peptide could be used as a model, with similar tests run. By mutating certain residues into nonreactive residues such as glycine and alanine a greater understanding of their importance to the interaction can be gained. For example, if the cysteine residue was replaced with an alanine residue mass spectrometry could be used to see if the peptide is platinated to the same extent. This change would illustrate the importance of cysteine to the interaction between cisplatin and the peptide. An identical 2D HSQC ^{1}H-^{15}N NMR experiment could be conducted to see if the complex retains its stability without the histidine residues. Finally, the VDI sequence could be altered completely and partially to further understand the significance of the residues to the interaction. The proposed experiments regarding individual residues could result in a better understanding of the interaction between cisplatin and hCTR1, as well as the cellular uptake mechanism of cisplatin.

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