The Reaction of a Platinated Methionine Motif of CTR1 with Cysteine and Histidine Is Dependent upon the Type of Precursor Platinum Complex ^[a]

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^[a] We dedicate this paper to Professor Giovanni Natile on the occasion of his 70th birthday.

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Abstract

The human copper protein (hCTR1) is believed to facilitate the cellular uptake of cisplatin. Cisplatin likely binds to the Methionine (Met)-rich motifs located in the N-terminus of hCTR1, and ligand exchange would be essential if cisplatin has to pass through the hCTR1 channel. In this work, we investigated the reaction between platinated adducts of a methionine-rich motif of yeast CTR1 (Mets7) and N-acetyl-cysteine (AcCys) or N-acetyl-histidine (AcHis), mimicking metal-binding residues downstream the CTR1 channel. Platination involved two cis-compounds, cisplatin and oxaliplatin, and one monofunctional complex, *cis*-diammine(pyridine)chloridoplatinum(II) (cDPCP). The reactions were monitored by HPLC and the products were characterized by ESI-MS. The results indicate different reactivity depending upon the platinum complex. The cisplatin/Mets7 adduct reacts readily with both cysteine and histidine ($t_{1/2} < 2$ min). In contrast, the oxaliplatin/Mets7 adduct reacts with cysteine but not with histidine, whereas cDPCP/Mets7 adduct reacts with histidine but not with cysteine. Hence, Mets7 adducts of these platinum complexes exhibit different reactivity towards downstream coordinating amino acids. These results suggest that each platinum complex possesses different reactivity and consequently may lead to differences in their cellular distribution and bioactivity.

Keywords: Copper transport proteins; Platinum Complexes; Anticancer drugs; Metal coordination; Substitution reactions.

Introduction

Platinum based anticancer drugs, including cisplatin, carboplatin, and oxaliplatin are widely used in clinic for the cancer chemotherapy.¹ It is generally agreed that platinum drugs target nuclear DNA and the DNA damage induces apoptosis.² However, the cellular uptake of platinum drugs is still not fully understood.³ Initially, it has been proposed that cisplatin, as a neutral molecule, could cross the cell membrane via passive diffusion and then be activated by intracellular hydrolysis.² More recently, increasing evidence indicates that also the human copper transport protein (hCTR1) could be involved in the cellular influx of cisplatin.⁴⁻⁶ Knockout of CTR1 reduces the uptake of cisplatin, resulting in drug resistance; its re-expression recovers the drug sensitivity.⁶ Treatment with cisplatin down-regulates CTR1, leading to drug resistance.^{7,8} It has also been shown that using copper chelators to reduce the expression of CTR1 can enhance cisplatin efficacy.^{9,10}

While more evidence supports that hCTR1 facilitates the cellular uptake of cisplatin, it is still controversial whether hCTR1 is also involved in the transport of oxaliplatin.^{11,12} On the other hand, the organic cation transporters (OCT) were found to mediate the cellular uptake of oxaliplatin and *cis*-diammine(pyridine)chloridoplatinum (cDPCP).¹³ After entering cells, platinum drugs are delivered to various subcellular compartments.^{3,14} Proteins play crucial roles also in these processes.¹⁵

hCTR1 is a glycosylated membrane protein (190 aa) with three putative transmembrane domains. The extracellular N-terminus contains two Met-rich and two His-rich motifs that are essential for the copper binding.¹⁶ These copper binding motifs are found also to have high affinity for cisplatin. Studies on a Met-rich motif of yeast CTR1 (Mets7) showed that cisplatin and transplatin exhibit different binding mode, the former loosing also the ammine ligands which, instead, are kept by the latter.^{17,18} Further studies on larger extracellular domains of hCTR1 confirmed the binding to methionine residues,¹⁹ and a kinetic study performed on various mutants indicated that the substitution rate is correlated to the number of methionine residues in the domain.²⁰

The binding of platinum complexes to the extracellular domains is likely the first step in the cellular uptake associated to hCTR1. The transmembrane domain and the intracellular domain of hCTR1 also contain metal binding residues (histidine, methionine, and cysteine) and these residues are proposed to facilitate copper transport via coordination transfer.^{16,21,22} By analogy, it has been proposed that also cisplatin could enter cells via hCTR1 through a methionine-based sulfur-sulfur exchange.²⁰ However, it is still unknown whether platinum bound to Met-rich motifs of hCTR1 is still reactive towards downstream coordinating amino acids and how kinetically favorable is the platinum transfer. In this work, we investigated the reaction of a platinated Met-rich motif of CTR1 with cysteine and histidine, two downstream coordinating amino acids of hCTR1. The reactions were performed on two *cis*-coordinated platinum drugs, cisplatin and oxaliplatin, and a monofunctional antitumor platinum complex, cDPCP.²³ Results indicate that the reactivity of the platinated Met-rich motif is dependent upon the type of precursor complex. The

cisplatin adduct reacts with both the cysteine and the histidine, the oxaliplatin adduct reacts with the cysteine but not with the histidine, finally, the cDPCP adduct reacts with histidine but not with cysteine. Moreover, the am(m)ine ligands are lost in the case of cisplatin but kept (at least in part) in the case of oxaliplatin and cDPCP. These data suggest that hCTR1 could have different functions towards different platinum complexes.

2. Results

2.1 The reaction of the cisplatin/Mets7 adduct

2.1.1 Reaction of cisplatin with Mets7

The reaction of cisplatin with Mets7 has been investigated by the Natile's group.¹⁷ Herein the reaction was monitored by HPLC. Two major cisplatin/Mets7 adduct species (peaks A1 and A2) were observed on the HPLC profile at retention time of 6.9 and 8.5 min, respectively, and these two peaks remain stable for over 20 h. ESI-MS spectra indicate the presence of two major end products, $[\text{Pt}(\text{Mets7})\text{Cl}+\text{H}]^{2+}$ ($m/z = 557.62$) and $[\text{Pt}(\text{Mets7})]^{2+}$ ($m/z = 539.64$). The hydrolyzed product, $[\text{Pt}(\text{Mets7})(\text{H}_2\text{O})]^{2+}$ ($m/z = 548.09$), was also present as a minor species in the MS spectra. This result is consistent with previous reports that binding of cisplatin to Mets7 leads to the release of ammine ligands.¹⁷ The cisplatin/Mets7 adduct species were used as reactants in the subsequent experiments.

2.1.2 Reaction of the cisplatin/Mets7 adduct with AcHis

The reaction of the cisplatin/Mets7 adduct with 2-fold molar excess of *N*-Acetyl-L-Histidine (AcHis) was monitored by HPLC (Figure 2A). The cisplatin/Mets7 adduct species (A1 and A2) decreased by over 60% in 2 minutes, and disappeared completely in 40 minutes. Three major products were rapidly formed with retention times of 5.2, 7.2 and 7.5 min on the HPLC profile. The products at 40 minutes were directly analyzed by ESI-MS (Figure 2B). The ESI-MS spectrum shows three products: $[\text{Pt}(\text{Mets7})(\text{AcHis})]^{2+}$ ($m/z = 638.18$), $[\text{Pt}(\text{Mets7})(\text{AcHis})\text{Cl}+\text{H}]^{2+}$ ($m/z = 656.20$), and $[\text{Pt}(\text{Mets7})(\text{AcHis})_2]^{2+}$ ($m/z = 736.64$). This result indicates that the cisplatin/Mets7 adduct is highly reactive to AcHis and multiple products are formed in the reaction.

2.1.3 Reaction of the cisplatin/Mets7 adduct with AcCys

The reaction of the cisplatin/Mets7 adduct with *N*-Acetyl-L-Cysteine (AcCys), monitored by HPLC, is shown in Figure 3. The two platinated Mets7 adduct species decreased quickly while incubating with AcCys ($t_{1/2} < 2$ min). Four products appeared on the HPLC profile with retention times of 5.8, 6.2, 6.5, and 7.1 min, respectively. These products remained stable for the following 40 minutes; then slowly evolved to more products (10 hours reaction). However, ESI-MS spectra recorded at 40 minutes showed only one peak with m/z 621.12 (2+), which was assigned to $[\text{Pt}(\text{Mets7})(\text{AcCys})]^{2+}$ (Figure 3B). It is possible that different HPLC peaks have the same composition (identical m/z value) and differ for the binding mode of Mets7 to platinum. Notably, the HPLC peak of free Mets7 (retention time of 12.2 minutes) increases with the reaction time. ESI-MS spectra confirmed the formation of free Mets7 in the reaction ($m/z = 884.30$, 1+). This observation indicates that the reaction of AcCys leads to the slow release of the Mets7 peptide. Together with the previous data on the reaction with AcHis, these results indicate that cisplatin/Mets7 adduct is

highly reactive to both histidine and cysteine residues.

2.2 Reactions of the oxaliplatin/Mets7 adduct

2.2.1 Reaction of oxaliplatin with Mets7

The reaction of oxaliplatin with Mets7 was performed in a 1:1.5 molar ratio. The time dependent HPLC profiles show a decrease of oxaliplatin and free Mets7 peaks, while two major products (peaks B1 and B2 with retention time of 8-9 min) were formed (Figure 4A). B1 and B2 remain stable during the following 12 hours. Only one major signal (m/z 596.62, 2+) was observed in the ESI-MS spectrum of the reaction solution (Figure 4B). The ESI-MS signal corresponds to $[\text{Pt}(\text{DACH})(\text{Mets7})]^{2+}$. This result indicates that Mets7 substitutes the oxalate ligand of oxaliplatin, while the bidentate 1,2-diaminocyclohexane (DACH) ligand remains coordinated to platinum.²⁴ Most probably different methionine residues of Mets7 are involved in platinum coordination which explains the observation of multiple products in the HPLC profile.

2.2.2 Reaction of the oxaliplatin/Mets7 adduct with AcCys and AcHis

The reaction of the oxaliplatin/Mets7 adduct with 2-fold excess of AcCys or AcHis was monitored by HPLC (Figure 5). The reaction with AcCys caused the decrease of the peaks of oxaliplatin/Mets7 adduct species (B1 and B2), whilst three new peaks emerged with retention times of 8 - 9 min. The reaction was very fast (over 60% of oxaliplatin/Mets7 adduct reacted in 2 minutes). In addition, free Mets7 was slowly produced in the following 18 h. The products formed after 40 minutes reaction were analyzed by ESI-MS. The spectrum showed only one fragment at m/z 678.12 (2+), which was assigned to $[\text{Pt}(\text{DACH})(\text{Mets7})(\text{AcCys})]^{2+}$. This result indicates that AcCys can quickly bind to $[\text{Pt}(\text{DACH})(\text{Mets7})]$ possibly by the substitution of one coordinated end of Mets7. Furthermore, Mets7 can be slowly released if the reaction is performed in the presence of excess AcCys. In contrast, oxaliplatin/Mets7 did not react with AcHis; the HPLC profiles showed no change incubating the adduct with AcHis for 36 hours (Figure 5B).

2.3 Reactions of the cDPCP/Mets7 adduct

2.3.1 Reaction of cDPCP with Mets7

The reaction of cDPCP (*cis*- $[\text{Pt}(\text{NH}_3)(\text{Py})\text{Cl}]\text{Cl}$) with Mets7 (1:1.2 molar ratio) was performed at 25 °C in water at pH 7.0. HPLC profiles show that two major adduct species are formed with retention times of 7.3 (**C1**) and 6.2 min (**C2**) (Figure 6). The reaction half-life, based on the decline of concentration of cDPCP, was about 1 hour. The first product formed was (**C1**), while the second product (**C2**) appeared after 4 hours and increased with the reaction time. The two products (**C1** and **C2**) were separated and analyzed by ESI-MS. As shown in Figure 7, **C1** yielded one abundant fragment at m/z of 587.14 (2+), which was assigned to $[\text{Pt}(\text{NH}_3)(\text{Py})(\text{Mets7})]^{2+}$. The spectrum of **C2** also yielded an abundant fragment at m/z 587.14 (2+) and, in addition, two new, quite intense, fragments at m/z 749.54 and 788.56 corresponding to $\{\text{Mets7} + [\text{Pt}(\text{NH}_3)(\text{Py})] + [\text{Pt}(\text{NH}_3)(\text{Py})(\text{Cl})] - \text{H}\}^{2+}$ and $\{\text{Mets7} + [\text{Pt}(\text{NH}_3)(\text{Py})] + [\text{Pt}(\text{NH}_3)(\text{Py})(\text{TFA})] - \text{H}\}^{2+}$, (TFA = Trifluoroacetic acid) respectively. Therefore, **C2** was confidently assigned to a bis-platinated Mets7 species. The fact that an abundant fragment with m/z 587.14 was observed also in the ESI-MS of **C2** is probably due to the dissociation of one platinum moiety during the ESI-MS process.

It was also confirmed that, while **C1** is the major product in the reaction of cDPCP with Mets7 in 1:1.2 ratio, **C2** appears to be the major adduct if the cDPCP / Mets7 ratio is increased to 2:1 (data not shown). In both **C1** and **C2** products, only one ammine remains bound to the platinum core. Thus this result indicates that after binding to Mets7, one ammine ligand of cDPCP is lost (probably that *trans* to the chlorido ligand displaced by Mets7). This is in line with methionine exerting a strong *trans* effect.²⁵

2.3.2 Reaction of the cDPCP/Mets7 adduct with AcCys and AcHis

The reaction of cDPCP/Mets7 adduct species **C1**, $[\text{Pt}(\text{NH}_3)(\text{Py})(\text{Mets7})]^{2+}$ with equimolar AcHis or AcCys was investigated in aqueous solution at pH 7.0. HPLC profiles show that **C1** reacts with AcHis rather quickly (~80% decrease of intensity after two minutes) (Figure 7A). Meanwhile, four product peaks emerged with retention times of 5.8, 7.8, 9.0, and 9.6 min, respectively. These products were stable for the following 12 hours. The products formed after 40 minutes incubation were analyzed by ESI-MS. The spectrum showed only one signal at m/z 686.12 (2+), which was identified as $[\text{Pt}(\text{NH}_3)(\text{Py})(\text{Mets7})(\text{AcHis})]^{2+}$. This result indicates that AcHis coordinates to Pt probably displacing one coordinated end of Mets7, which probably becomes mono-coordinated to platinum. The presence in the HPLC profile of various peaks related to product formation indicates that different isomers are formed.

The reaction of **C1** with equimolar AcCys was also monitored by HPLC at pH 7.0. The peak of **C1** and AcCys remained unchanged for 12 hours (Figure 8). This result indicates that *trans*- $[\text{Pt}(\text{NH}_3)(\text{Py})(\text{Mets7})]$ is unreactive towards AcCys.

Discussion:

It has been recognized that DNA is the ultimate target of platinum anticancer drugs which can form fatal DNA-Pt crosslinks.²⁶ However, for targeting nuclear DNA, cisplatin has to pass through the cell membrane, cytoplasmic compartments, and finally reach the nucleus. It is estimated that, within cells, only ~1% of cellular platinum binds to nuclear DNA.²⁷ Proteins have been shown to play important roles in determining the cytotoxicity and drug resistances of platinum agents. Particularly, proteins associated with copper homeostasis in cells, such as CTR1, Atox1, Cox17 and ATPase, have been found to influence cellular uptake, distribution and excretion of cisplatin.^{9,28} In addition, cytoplasmic glutathione, metallothioneins, and other thiol-containing biomolecules are capable to sequester platinum drugs due to the high binding affinity of sulfur for platinum. Thus sulfur compounds are able to deactivate platinum drugs and increase resistance to the treatment.^{29,30} Furthermore, platinum complexes may also bind to transcription factors containing zinc finger domains, such as metal-responsive MTF-1 and human transcription factor Sp1.^{29,31} These findings furthermore stress the importance of mechanisms related to transport and distribution in cells in determining the pharmacology of platinum drugs.

In this work, we investigated the reaction of a selection of platinum drugs with a Met-rich motif of yeast CTR1 and, after characterization of the relevant adduct species, we tested their reactivity with cysteine and histidine, two downstream

coordinating amino acids present in the CTR1 channel. The three platinum drugs were selected not only for their clinical relevance, but also for their structural diversity: cisplatin has monodentate ammine ligands; oxaliplatin has *cis* configuration as cisplatin, but contains a chelated diamine ligand (DACH); finally, cDPCP has two *cis* ammines as cisplatin but, in addition, a pyridine so that it has only one labile chlorido ligand.

The reaction with Mets7 gives, in the three cases, three different types of adducts: displacement of the two ammines in cisplatin, retention of the diamine in oxaliplatin, conservation of only one ammine and its *trans* pyridine in the case of cDPCP. The formation of three different products can be accounted for on the basis of the trans-labilizing effect of S-donor methionines and extra stabilization of chelated ligands (chelate effect). Thus in cisplatin, after substitution by Mets7 of labile chlorido/aqua ligands, the trans-labilized ammines are readily displaced by other methionines of Mets7. It is rather curious that, while there is a platinum species still containing chloride (a labile ligand) there is no ESI-MS fragment containing ammine(s). Although we are aware of the fact that care must be used when correlating gas-phase ions to species present in solution,³² we believe that the stability of the chlorido species is increased by partial charge neutralization of otherwise doubly charged complex.

In the case of oxaliplatin, after substitution of the labile ligand by Mets7, notwithstanding the trans-labilizing effect of coordinated methionines, the DACH ligand remains firmly bound to platinum owing the contribution to stability of the chelate effect. In the case of cDPCP, after substitution by a Mets7 methionine of the single labile ligand (a chloride), the trans-labilizing effect of the S-donor ligand promotes labilization of the *trans* ligand (an ammine). In the formed product Mets7 spans *trans* positions with two coordinated methionines. The third methionine can coordinate a second platinum fragment in which the ammine *trans* to methionine has also been labilized and its position has been taken by a chloride or a trifluoroacetate (the latter present in the solution for ESI-MS). All above reactions take place in terms of minutes.

The subsequent reactions between platinum/Mets7 derivatives and AcCys or AcHis were performed to investigate the reactivity of a platinum complex already bound to a Met-rich motif. Cys and His are good ligands for platinum and are present downstream the CTR1 channel from the extracellular N-terminus to the intracellular C-terminus. The cisplatin/Mets7 derivatives, in which three coordination positions are saturated by methionines and there is still a labile ligand (chloride or water), react readily with both AcCys and AcHis (<2 min). In the case of His, apart from the product containing only one His, are also present species containing one His and one chloride or two His. This is a clear indication that there is some strain in the tridentate macrochelate which can cause its switch to a bidentate ligand. Moreover, chloride coordination can be favored by partial neutralization of the 2+ charge of the metal core while coordination of a second His may be favored by mutual labilization of Mets7 methionines when placed in *trans* to one another (His will compete less with the *trans* methionine than a second S-donor ligand). In the case of reaction with

AcCys, the only observed platinum product is that containing Mets7 and one Cys plus formation of significant amount of free Mets7 at longer time (40 min). The release of Mets7 is likely favored by mutual labilization of *trans* S-donors (Mets7' methionines and cysteine).

Differently from the previous case, the oxaliplatin-Mets7 derivatives, containing chelated DACH and bidentate Mets7, reacts only with AcCys while no reaction with AcHis is observed even after 12 h incubation. Moreover, the only platinum species observed in ESI-MS contains one Cys in addition to DACH and Mets7. The formation of such a species may be favored despite some steric strain in a *cis*-chelated macrochelate formed by Mets7 (at least a 17-membered chelate ring) and partial charge neutralization consequent to coordination of a Cys in its deprotonated form. The DACH ligand remains firmly bound to platinum.

The reactivity of cDPCP-Mets7 derivatives towards AcCys and AcHis was also different from those observed in the previous two cases. The platinum adduct containing one ammine and a pyridine (*trans* to one another) and a Mets7 spanning two *trans* coordination positions reacted only with His but not with Cys. This might be an indication that Mets7 spanning two *trans* positions is less strained than one which is *cis*-chelated; as a consequence, in the case of Cys the mutual *trans*-labilization of S-donors disfavors the adduct with Cys coordinated *trans* to a monodentate Mets7 with respect to a *trans*-chelated bidentate Mets7. Such a mutual labilization of *trans* ligands does not apply in the case of His coordinated *trans* to a monodentate Mets7 and the corresponding adduct is formed.

It is noteworthy that all reactions of platinum/Mets7 adducts with His and Cys occur rapidly (few minutes) and even when no new products are formed (as in the case of oxaliplatin/Mets7 adducts with His and cDPCP/Mets7 adducts with Cys) we are inclined to think that this is under thermodynamic rather than kinetic control.

Furthermore, except in the case of cisplatin in which both ammines are displaced by Mets7, in the other two cases the N-donor ligands (DACH in one case and *trans*-ammine-pyridine in the other case) are kept throughout the different steps. Therefore, except in the case of cisplatin, in the other two cases an active platinum species still carrying ammine ligands could be able to enter the cell through a CTR1 pathway. If this is the case in the real mechanism, remains to be proved.

Interestingly, classical concepts in inorganic chemistry, such as *trans*-effect, chelation effect, mutual labilization of *trans* ligands and steric strain in a macro chelate fully apply also to the case of interaction with biologically relevant substrates. It is also shown that large chelate rings are better accommodated by coordination in *trans* positions.³³ Here in this work, Platinum/Mets7 adducts exhibiting different reactivity towards amino acids (histidine and cysteine) helps understand the different cellular distribution and bioactivity of various platinum complexes. Other copper proteins, such as Atox1, Cox17 and ATPase, have also been demonstrated to modulate the cellular distribution and excretion of cisplatin.^{9,15,28} Atox1 containing metal-binding motif (CXXC), which is highly reactive to cisplatin.³⁴ Recent studies show that

platinum can transfer between proteins, such as from Atox1 to the downstream protein ATPase.³⁵⁻³⁷ Cox17, a copper chaperone for mitochondria, is able to facilitate the cisplatin accumulation in mitochondria,¹⁵ in which the platinum transfer between proteins probably occurs. The different reactivity of platinum/Mets7 adducts offers an implication that various platinum agents could exhibit significant platinum transfer efficiency, which consequently influence the cellular distribution of different platinum complexes.

3. Conclusions

In summary, a methionine-rich motif (Mets7) of the copper transporter CTR1 has been reacted with different platinum complexes, including two clinically relevant drugs, cisplatin and oxaliplatin, and a monofunctional complex, cis-diammine(pyridine)chloridoplatinum(II) (cDPCP). The characterized platinum/Mets7 adducts have then been reacted with N-acetyl-cysteine and N-acetyl-histidine, two platinum-coordinating amino acids present downstream the CTR1 channel. Different reactivity has been observed depending upon the type of platinum drug. The cisplatin/Mets7 adduct is highly reactive to both cysteine and histidine. The oxaliplatin/Mets7 adduct is reactive only to cysteine but not to histidine, finally, the cDPCP/Mets7 adduct is reactive only to histidine but not to cysteine. These results indicate that the various platinum complexes have different reactivity towards amino acids downstream the CTR1 channel. All reactions of platinum/Mets7 adducts are fast ($t_{1/2} < 2$ min) and, except in the case of cisplatin, N-donor ligands can be kept throughout the reaction steps. Therefore, except in the case of cisplatin, in the other two cases an active platinum species still carrying the am(m)ine ligands could be able to enter the cell through a CTR1 pathway. If this is the case in the real mechanism, remains to be proved. These data also indicate that even quite similar platinum complexes can have very different reactivity towards proteins. These differences could be correlated with the cellular distribution and the bioactivity of these platinum agents.

4. Experimental Section

Materials:

The N-terminal acetylated peptide Mets7 (AcMTGMKGMS) was synthesized by standard Fmoc chemistry. The crude peptide was purified using HPLC and the identity of the peptide was verified by ESI-MS. Cisplatin and oxaliplatin were purchased from Sigma-Aldrich. *cis*-[Pt(NH₃)₂(Py)Cl]Cl (cDPCP) was prepared according to the literature.^{38,39} N-Acetyl-L-Cysteine (AcCys) and N-Acetyl-L-Histidine (AcHis) were purchased from BIO BASIC INC. Acetonitrile and methanol were chromatographic grade reagents.

Preparation of platinum/Mets7 adducts:

Cisplatin, oxaliplatin, cDPCP, Mets7, AcHis and AcCys were directly dissolved in ultra-pure H₂O as stock solutions. The pH of all starting solutions was adjusted to 7.0 with HCl and NaOH before reactions. For the preparation of platinum/Mets7 adducts, cisplatin, oxaliplatin and cDPCP were reacted with Mets7 (1:1.2 or 1:1.5-molar ratio at 25 °C and pH 7.0). The concentration of platinum complexes was 1-2 mM. Aliquots

of the reaction solution were withdrawn at different times, frozen at -80 °C, and subsequently analyzed by HPLC and ESI-MS.

HPLC analysis:

High pressure liquid chromatography (HPLC) was performed on an Agilent 1200 system with UV detection at 220 nm. A reverse phase Kromasil C18 column (250×4.6mm, 5 μm) was used. The mobile phase A was 0.1% trifluoroacetic acid (TFA) solution and B was 100% methanol. Linear gradient was used from 30% to 90% of eluent B in 20 minutes at a flow rate of 1.0 mL/min. For ESI-MS samples preparation, aliquots from reaction solution or HPLC samples were immediately frozen and lyophilized.

Electrospray Ionization Mass Spectrometry (ESI-MS):

Mass spectrometric measurements were performed on a Thermo LTQ linear ion trap mass spectrometer (Thermo Fisher, San Jose, CA, USA). The mobile phase of ESI-MS was 1:1 water/methanol containing 0.1% formic acid. The reaction samples were diluted in the mobile phase and directly infused at a flow rate of 3 μL·min⁻¹. The ESI-MS spectra were detected in positive mode, with spray voltage of 1.8 kV and capillary voltage of 32 V. The capillary temperature was kept at 200 °C. Spectra were acquired and analyzed by Xcalibur 2.0 (Thermo).

Table of Abbreviations

cDDP	cisplatin
oxPt	oxaliplatin
cDPCP	<i>cis</i> -diammine(pyridine)chloridoplatinum(II)
Py	Pyridine
AcHis	N-Acetyl-L-Histidine
AcCys	N-Acetyl-L-Cysteine
TFA	Trifluoroacetic acid

Acknowledgments

This work was supported by the National Science Foundation of China (U1332210, 21171156), the National Basic Research Program of China (973 Program, 2012CB932502), the Ministry of Education of China (SRFDP 20133402110041) and the Collaborative Innovation Center of Suzhou Nano Science and Technology. This research was carried out within the framework of the European Union CMST COST Action CM1105 “Functional metal complexes that bind to biomolecules” and the Inter-University Consortium for Research on the Chemistry of Metal Ions in Biological Systems (CIRCMSB, Bari, Italy).

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Figure/Scheme Legends

Scheme 1. Structure formulae of cisplatin, oxaliplatin and cDPCP.

Figure 1. (A) Reaction of cisplatin with Mets7 (1:1 molar ratio) monitored by HPLC in water at pH 7.0. (B) ESI-MS characterization of the final products at 20 hours. cDDP denotes cisplatin.

Figure 2 Reaction of the cisplatin/Mets7 adduct with 2-fold molar excess of AcHis. (A) HPLC profiles at different reaction times; (B) ESI-MS analysis of products after 40 minutes reaction.

Figure 3 Reaction of the cisplatin/Mets7 adduct with 2-fold excess of AcCys performed in water at pH 7.0. (A) HPLC profiles at different reaction times. (B) Comparison between ESI-MS spectra of the cisplatin/Mets7 adduct before and after reaction with AcCys for 40 minutes.

Scheme 2. Proposed reaction scheme for the formation of cisplatin/Mets7 adduct species and their reactions with AcHis and AcCys. Y denotes a coordination site (N or O) of Mets7.

Figure 4. (A) HPLC traces of the reaction of oxaliplatin with Mets7 (molar ratio 1:1.5) in water at pH 7.0. (B) ESI-MS identification of oxaliplatin/Mets7 adducts. oxPt denotes oxaliplatin.

Figure 5. HPLC monitoring of the reactions of oxaliplatin/Mets7 adduct with 2-fold molar ratio of AcCys (A) and AcHis (B) in water solution, pH 7.0. (C) ESI-MS analysis of the products formed in the reaction of oxaliplatin/Mets7 adduct with AcCys after 40 minutes.

Scheme 3 Proposed reaction scheme for the formation of oxaliplatin/Mets7 adduct species and their reactions with AcCys or AcHis.

Figure 6 (A) HPLC profiles of the reaction of cDPCP with Mets7 (1:1.2 molar ratio) at pH 7.0 and 25 °C. (B) ESI-MS analysis of products (C1) and (C2) isolated by HPLC.

Figure 7. (A) HPLC monitoring of the reaction of the cDPCP/Mets7 adduct **C1** $[\text{Pt}(\text{NH}_3)(\text{Py})(\text{Mets7})]^{2+}$ with AcHis (1:2 molar ratio) at pH 7.0. (B) ESI-MS spectrum of the reaction products after 40 minutes incubation.

Figure 8. HPLC monitoring of the reaction between the cDPCP/Mets7 adduct (**C1** $[\text{Pt}(\text{NH}_3)(\text{Py})(\text{Mets7})]^{2+}$) and equimolar AcCys at pH 7.0.

Scheme 4. Proposed reaction scheme for the formation of cDPCP/Mets7 adduct species and their reactions with AcHis or AcCys.

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Graphical Abstract