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The reaction of a platinated methionine motif of CTR1 with cysteine and histidine is dependent upon the type of precursor platinum complex☆

Guolin Ma [a,1], Qin Wu [a,1], Xuelei Wu [a], Fabio Arnesano [b], Giovanni Natile [b], Einar Sletten [c], Yangzhong Liu [a,*]

[a] CAS High Magnetic Field Laboratory, Department of Chemistry, University of Science and Technology of China, Hefei Anhui 230026, China
[b] Department of Chemistry, University of Bari "A. Moro", via E. Orabona 4, 70125 Bari, Italy
[c] Department of Chemistry, University of Bergen, Norway

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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 reactivities 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 reactivities towards downstream coordinating amino acids. These results suggest that each platinum complex possesses different reactivities and consequently may lead to differences in their cellular distribution and bioactivity.

1. 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]. (See Schemes 1.)

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 modes, 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

☆ We (GM, QW, XW, FA, ES and YL) dedicate this paper to Professor Giovanni Natile on the occasion of his 70th birthday.

* Corresponding author.
E-mail address: liuyz@ustc.edu.cn (Y. Liu).

[1] These authors contributed equally to this work.

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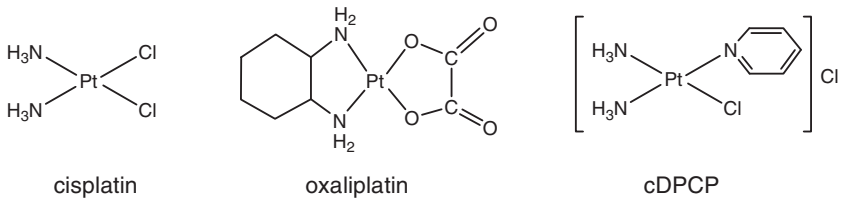


Scheme 1. Structure formulae of cisplatin, oxaliplatin and cDPCP.

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 last 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

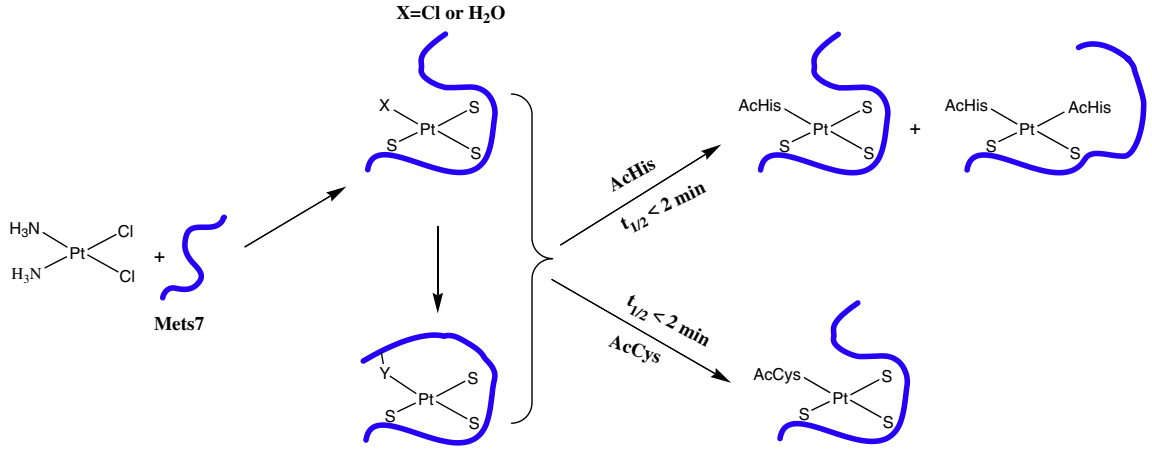
The reaction of cisplatin with Mets7 has been investigated by the Natile's group [17]. Herein the reaction was monitored by HPLC. Two major cisplatin/Mets7 adduct species (peaks A1 and A2) were observed on the HPLC profile at retention times of 6.9 and 8.5 min, respectively, and these two peaks remain stable for over 20 h. ESI-MS spectra indicate the presence of two major end products, $[Pt(Mets7)Cl + H]^{2+}$ (m/z = 557.62) and $[Pt(Mets7)]^{2+}$ (m/z = 539.64). The hydrolyzed product, $[Pt(Mets7)(H_2O)]^{2+}$ (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 (Fig. 2A). The cisplatin/Mets7 adduct species (A1 and A2) decreased by over 60% in 2 min, and disappeared completely in 40 min. 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 min were directly analyzed by ESI-MS (Fig. 2B). The ESI-MS spectrum shows three products: $[Pt(Mets7)(AcHis)]^{2+}$ (m/z = 638.18), $[Pt(Mets7)(AcHis)Cl + H]^{2+}$ (m/z = 656.20), and $[Pt(Mets7)(AcHis)_2]^{2+}$ (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. (See Fig. 1.)

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 Fig. 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 min; then slowly evolved to more products (10 hour reaction). However, ESI-MS spectra recorded at 40 min showed only one peak with m/z 621.12 (2+), which was assigned to $[Pt(Mets7)(AcCys)]^{2+}$ (Fig. 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 min) increases with the reaction time. ESI-MS spectra confirmed the formation of free Mets7 in the



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.

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