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Fast cleavage of a diselenide induced by a platinum(II)–methionine complex and its biological implications

Qin Liu [a,c], Xiaoyong Wang [b,*], Xiaoliang Yang [c], Xiao Liang [c], Zijian Guo [c,*]

[a] Applied Chemistry Department, Nanjing University of Finance and Economics, Nanjing 210003, PR China
[b] State Key Laboratory of Pharmaceutical Biotechnology, School of Life Sciences, Nanjing University, Nanjing 210093, PR China
[c] State Key Laboratory of Coordination Chemistry, School of Chemistry and Chemical Engineering, Nanjing University, Nanjing 210093, PR China

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ABSTRACT

Platinum-based anticancer drugs such as cisplatin induce increased oxidative stress and oxidative damage of DNA and other cellular components, while selenium plays an important role in the antioxidant defense system. In this study, the interaction between a platinum(II) methionine (Met) complex [$Pt(Met)Cl_2$] and a diselenide compound selenocystine [$(Sec)_2$] was studied by electrospray ionization mass spectrometry, high performance liquid chromatography mass spectrometry, and ^1H NMR spectroscopy. The results demonstrate that the diselenide bond in $(Sec)_2$ can readily and quickly be cleaved by the platinum complex. Formation of the selenocysteine (Sec) bridged dinuclear complex $[Pt_2(Met\text{-}S,N)_2(\mu\text{-}Sec\text{-}Se,Cl)]^{3+}$ and Sec chelated species $[Pt(Met\text{-}S,N)(Sec\text{-}Se,N)]^{2+}$ was identified at neutral and acidic media, which seems to result from the intermediate $[Pt(Met\text{-}S,N)(Sec\text{-}Se)Cl]^{+}$. An accelerated formation of S–Se and S–S bonds was also observed when $(Sec)_2$ reacted with excessive glutathione in the presence of $[Pt(Met)Cl_2]$. These results imply that the mechanism of activity and toxicity of platinum drugs may be related to their fast reaction with seleno-containing biomolecules, and the chemoprotective property of selenium agents against cisplatin-induced toxicity could also be connected with such reactions.

1. Introduction

Platinum anticancer drugs such as cisplatin and oxaliplatin have been widely used in clinical chemotherapy to treat diverse malignancies [1]. The major cytostatic mechanism of these drugs is believed to form irreversible Pt–DNA adducts through covalent cross-linking with DNA [2]. However, the molecular process of platinum drugs *in vivo* is not merely limited to ligand substitution by DNA; similar reactions could proceed with other biomolecules containing nucleophilic groups such as thiols and selenols, which are vital for many enzyme activities [3,4]. For example, mammalian thioredoxin reductase (TrxR), a selenoprotein containing a selenocysteine (Sec) residue in its active site [5], is one of such targets. Electrophilic compounds, such as cisplatin and its glutathione (GSH) adduct [6], oxaliplatin and some other platinum(II) complexes [7–9], can easily target TrxR and inactivate the enzyme, which would disturb the function and viability of cells [10]. In fact, cisplatin-modified TrxR can be a potent and direct trigger of apoptosis in cancer cells [11].

Sec residue is also found in the active site of glutathione peroxidases (GPxs) and other selenoproteins. Nevertheless, low pK_a value (5.3) and strong nucleophilicity of Sec make it highly reactive and unable to exist freely in cells [12]. As a result, many Sec residues in enzymes form transient selenenylsulfide bonds with proximal cysteine residues as part of their catalytic mechanisms. Recently, natural selenoproteins containing a diselenide bond formed by two Sec residues have also been identified [13]. Oxidoreductases containing either selenenylsulfide or diselenide bonds can have physiologically compatible redox potentials and enhanced reduction kinetics in comparison with their sulfide counterparts, which may influence the catalysis of selenoenzymes [14]. Even diselenide bond in selenoglutathione (GSeSeG) is able to oxidize the common biological cofactor NADPH and the cysteine residue in unfold proteins [15]. For these reasons, studies on diselenide bond have elicited great interests in recent years [16,17]. However, to the best of our knowledge, little attention has been paid to the interactions of platinum drugs with seleno-containing biomolecules besides our own research [18–20]. Such studies may unveil some hidden facts underlying the therapeutic and protective mechanism for platinum anticancer drugs.

In this paper, the interaction between a cisplatin model compound [$Pt(Met)Cl_2$] and selenocystine [$(Sec)_2$] (Fig. 1) was studied by electrospray ionization mass spectrometry (ESI-MS), ^1H NMR spectroscopy, and high performance liquid chromatography mass spectrometry (HPLC/MS). The fast cleavage of the Se–Se bond induced by [$Pt(Met)Cl_2$]

* Corresponding authors. Wang is to be contacted at State Key Laboratory of Pharmaceutical Biotechnology, School of Life Sciences, Nanjing University, Nanjing 210093, PR China. Tel.: +86 25 83594549; fax: +86 25 83314502. Guo, State Key Laboratory of Coordination Chemistry, School of Chemistry and Chemical Engineering, Nanjing University, Nanjing 210093, PR China. Tel.: +86 25 83594549; fax: +86 25 83314502.
E-mail addresses: boxwxy@nju.edu.cn (X. Wang), zguo@nju.edu.cn (Z. Guo).

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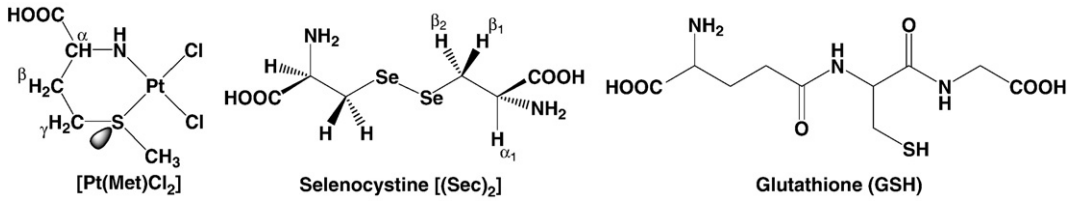

Fig. 1. Chemical structures of the compounds studied in this work.

and the formation of the Sec bridged dinuclear platinum intermediate have been detected. Since the major intracellular metabolite of cisplatin is the GSH adduct, GS–Pt [21], the impact of $[Pt(Met)Cl_2]$ on $(Sec)_2$ was also studied in the presence of GSH. The results indicate that even a trace amount of platinum complex can accelerate the cleavage of Se–Se bond under this condition. The biological implications of these findings were also discussed. We chose $[Pt(Met)Cl_2]$ and $(Sec)_2$ as model compounds to study the interaction because cisplatin is liable to release ammines when reacting with sulfur- or seleno-containing molecules and Sec is extremely unstable. Other complexes more similar to cisplatin like [Pt(DACH)Cl_2] and [Pt(en)Cl_2] directly form dinuclear platinum complexes when they reacted with GSH or GSSG [22,23], which may conceal the details about the reaction mechanism.

2. Experimental

2.1. Materials

L-Selenocystine [$(Sec)_2$], glutathione (GSH) and L-methionine (Met) were purchased from Sigma-Aldrich. K_2PtCl_4 was purchased from Shandong Boyuan Chemical Co., Ltd.; and methanol of HPLC grade used in ESI-MS and HPLC/MS was from Acros Organics. $[Pt(Met)Cl_2]$ was synthesized using a published method [24]. Double distilled water was prepared on a Mili-Q Academic system.

2.2. ESI-MS method

ESI-MS dada were acquired by an Agilent 1100 HPLC equipment coupled with a MSD (SL) ion-trap electrospray ionization mass spectrometer system. The reaction mixture (5 μL) of $[Pt(Met)Cl_2]$ with $(Sec)_2$, or with $(Sec)_2$ plus GSH was loaded after different reaction time by an auto sample injector. The sample was introduced to the mass analyzer by the mobile phase solution (MeOH:H_2O, 1:1) at a flow rate of 200 μL min^{-1}. The voltage used at the electrospray needles was 3.5 kV, and the capillary temperature was 350 °C. The mass spectra were recorded in the negative mode and positive mode respectively. The isotopic distribution pattern for each complex was simulated with the Isopro 3.0 program.

2.3. HPLC/MS method

The reaction mixture (5 μL) of $[Pt(Met)Cl_2]$ with $(Sec)_2$ was loaded onto the RP 18 column (150 mm × 4.6 mm, 5 μm; Agilent) by an autosampler and eluted with the mobile phase consisting of water and MeOH (4:1) at a flow rate of 0.4 mL min^{-1}. A T split was used before introducing the sample into the mass analyzer in order to reduce the flow. The experimental condition of the mass spectrometry was the same as described above.

2.4. NMR method

Samples for ^1H NMR experiments were prepared in a mixture of 90% water and 10% D_2O, and the pH was adjusted by NaOH (0.1 M) and HCl (0.1 M). The concentration of the reactants was consistent with that of the samples used in the ESI-MS experiments. The time-dependent ^1H NMR spectra were acquired on a Bruker DRX 500 MHz spectrometer using standard pulse sequences.

2.5. Reaction between $[Pt(Met)Cl_2]$ and $(Sec)_2$

Freshly prepared $[Pt(Met)Cl_2]$ (6 mM) was adjusted to an appropriate pH with NaOH or HCl (0.1 M). $(Sec)_2$ (1.0 mg) was added to double distilled water (1 mL) and the pH was adjusted by NaOH (0.1 M) until $(Sec)_2$ was dissolved, which resulted in a solution with a concentration of 3 mM and pH 7.0–7.5. The above two solutions were mixed together at a molar ratio of 2:1 and incubated at 25 °C. The pH of the solutions was measured again 30 min after the incubation to ascertain the completion of the reaction.

2.6. Reaction between $[Pt(Met)Cl_2]$ and $(Sec)_2$ in the presence of GSH

$(Sec)_2$ (200 μL, 1 mM) was mixed with GSH (200 μL, 2 mM) and incubated at 37 °C. The time-dependent ESI-MS spectra of the reaction in the presence and absence of $[Pt(Met)Cl_2]$ were recorded respectively. The effect of pH on the reaction was also evaluated by adjusting the pH of GSH solution before mixing with $(Sec)_2$ and measuring the pH of the mixture 30 min after the reaction.

3. Results

3.1. Reaction between $[Pt(Met)Cl_2]$ and $(Sec)_2$

3.1.1. ESI-MS results

The reaction between $[Pt(Met)Cl_2]$ and $(Sec)_2$ at a molar ratio of 2:1 and pH 7.02 was first studied by ESI-MS. Fig. 2 shows the spectra of the reaction mixture recorded after different incubation time at room temperature. A major peak at m/z 888.1 was observed only 5 min after the reaction, which can be assigned to the bridged dinuclear species $[Pt_2(Met\text{-}S,N)_2(\mu\text{-}Sec\text{-}Se,Cl) - 4H]^-$. As the insets in Fig. 2 indicate, the isotopic distribution pattern of this peak consists well with the simulated result. The peaks at m/z 924.6 and 946.8 can also be attributed to the bridged dinuclear species $[Pt_2(Met\text{-}S,N)_2(\mu\text{-}Sec\text{-}Se,Cl) - 3H + Cl]^-$ and $[Pt_2(Met\text{-}S,N)_2(\mu\text{-}Sec\text{-}Se,Cl) - 4H + Cl + Na]^-$, respectively. The peaks observed at m/z 509.1 and 567.4 are assignable to the species $[Pt(Met\text{-}S,N)(Sec\text{-}Se,N) - 3H]^-$ and $[Pt(Met\text{-}S,N)(Sec\text{-}Se,N) - 3H^+ + Cl + Na]^-$, respectively. The peak corresponding to reactant $(Sec)_2$ can barely be recognized in this spectrum, while that to $[Pt(Met\text{-}S,N)Cl_2 - H]^-$ at m/z 414.3 remained observable till 30 min later. The intensity of the peak at m/z 888.1 decreased while that of the peak at m/z 509.1 increased with the time. Three new peaks at m/z 490.3, 526.7, and 585.6 were observed 3 h after the reaction, which could be assigned to species $[Pt(Met\text{-}S,N)_2 - 3H]^-$, $[Pt(Met\text{-}S,N)_2 - 2H + Cl]^-$, and $[Pt(Met\text{-}S,N)_2 - 2H + 2Cl + Na]^-$, respectively. The peak at m/z 526.7 started to increase from then on, and that at m/z 888.1 kept decreasing until it disappeared 5 h later. After reaction for 24 h, the peak at m/z of 526.7 became the major species and the peaks at m/z 490.3, 509.1, and 585.6 still remained. The peak appeared at m/z 643.9 is attributed to species $[Pt(Met\text{-}S,N)_2 - 2H + 3Cl + 2Na]^-$.

The reaction between $[Pt(Met)Cl_2]$ and $(Sec)_2$ at a molar ratio of 2:1 was also investigated at pH 2.82 by ESI-MS. As Fig. 3 shows, the