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Probing the interactions between cisplatin and essential amino acids using electrospray ionization mass spectrometry

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ABSTRACT

Cisplatin (*cis*-[$PtCl_2(NH_3)_2$]) is an important platinum-containing anticancer drug for treatment of solid malignancies. Probing the interactions between cisplatin and free amino acids are beneficial for investigation of its pharmacology and side effects. In this study, the interactions of twenty amino acids with cisplatin in water, acidic, neutral and basic solutions were studied using electrospray ionization mass spectrometry (ESI MS), respectively. Adducts of cisplatin and amino acids were recognized. It had been found that the reactions of cisplatin with amino acids depended on solution media and essential amino acids. Multiple tandem mass spectrometry was employed to probe the affinities of nucleophilic functional groups, such as side chains, α-NH_2 groups and α-COOH groups on amino acids for cisplatin in water. The results indicated that the chelated complexes formed were stable in aqueous solution. For free Cys, Met and His, their side chains had higher affinities than α-NH_2 groups. For free Ser, Thr, Asp, Glu, Asn and Gln amino acids, their α-NH_2 groups had higher affinities than the side chains and α-COOH groups. For Lys, Arg, Phe, Tyr and Trp, platinum may coordinate to their aromatic rings of the side chains or α-NH_2 groups. For other amino acids Gly, Ala, Val, Leu, Ile and Pro, their α-amino groups had higher affinities for cisplatin than α-COOH groups. These results provide the insights for understanding of the mechanism and side effects of cisplatin.

1. Introduction

Although DNA was thought to be the main target for cisplatin to induce cancer cell's apoptosis, cisplatin could also interact with many nucleophiles before reaching DNA, especially some sulfur- and nitrogen-containing compounds, such as amino acids, GSH, proteins, and so on [1–4]. These interactions not only play crucial roles in its anticancer action, pharmacokinetics and biodistribution, but also contribute to its severe toxic side effects and resistance [5–7]. Most recently, more and more researchers pay great attention to investigate the interactions between cisplatin and these biological nucleophiles.

Amino acids were the basic units of proteins and peptides, and their interactions with cisplatin were important for understanding of its action and side effects because of their affinities for platinum(II) compound [5,8,9]. Kröning et al. found that sulfur-containing amino acids could decrease cytotoxicity and uptake of cisplatin in renal tubule epithelial cell lines [10]. Besides, Barnham et al. found that L-methionine could increase the rate of reaction between 5′-guanosine monophosphate and cisplatin, while Vrana and Brabec found L-methionine could inhibit reaction of DNA with cisplatin [11,12]. Moreover, Li et al. found methionine can favor DNA platination by trans-coordinated platinum antitumor drugs [13].

In the past 20 years, researchers have already done a lot of investigations of the interactions between cisplatin and amino acids, especially methionine and cysteine. Heudi et al. found that even if in the presence of high concentration of NaCl, cisplatin could react with L-methionine, while Bose found it was similar for L-cysteine [8,14]. Using the polarizable continuum model, Zimmermann et al. explored the interactions between cisplatin and sulfur-containing amino acids [15]. Combining HPLC with NMR techniques, El-Khateeb et al. studied the reactions of cisplatin hydrolytes with methionine, cysteine and plasma ultrafiltrate [16]. Appleton et al.

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studied the reaction of *cis*-diamminediaquaplatinum(II) cation (*cis*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$) with histidine and related molecules under different pH using NMR technique, and found that different adducts were produced at different pH values [17]. Hadi et al. reported that dinuclear sulfur-bridged platinum (II) complex was formed in the reaction of *cis*-$[Pt(^{15}NH_3)_2(H_2O)_2]^{2+}$ with *N*-acetylcysteine using electrospray MS [18]. Bandu et al. used ESI MS/MS combining liquid chromatography to identify and characterize in vivo metabolites of cisplatin with methionine (Met), cysteine (Cys) and acetylcysteine amino acids in rat kidney cancer tissues [19]. Until now, great progress has been made in the studies of reactions of platinum drugs with free amino acids, while most of works mainly focused on Met, Cys and histidine (His) [20,21]. However, it has been reported that for the interactions of cisplatin with proteins and peptides, the other amino acid residues, such as aspartic acid (Asp), glutamic acid (Glu), threonine (Thr), serine (Ser), tyrosine (Tyr), arginine (Arg) and lysine (Lys) residues, were also the binding sites of cisplatin [5,22,23].

Here, the aim of this study is to gain insights into the reactions of cisplatin with twenty free amino acids in water, acidic, neutral and basic solutions using ESI MS. On the basis of the MS data, multiple adducts could be identified directly and the possible reaction pathways were proposed. The affinities of nucleophilic functional groups on amino acids for cisplatin were probed by MS^n.

2. Experimental

2.1. Materials

Milli-Q^{TM} water (18.2 MΩ) (Millipore, Bedford, MA) was used in all the experiments. His, Cys, glycine (Gly), alanine (Ala), valine (Val), leucine (Leu), isoleucine (Ile), Ser, Lys, Arg, proline (Pro), phenylalanine (Phe), Tyr and tryptophan (Trp) were bought from Sinopharm Chemical Reagent (Shanghai, China). Met, Glu, asparagine (Asp), glutamine (Gln), asparagine (Asn) and Thr were purchased from Huishi Biochemical Reagent (Shanghai, China). All amino acids were L-type. Cisplatin (purity 99.9%) was purchased from Sigma-Aldrich (St. Louis Missouri). Ammonium acetate (NH_4OAc) was acquired from Fluka (Buchs, Switzerland). Ammonium hydroxide (analytical grade) was bought from Beijing Chemical Work (Beijing, China). Formic acid (HPLC grade) was bought from TEDIA Company (Fairfield, OH). All chemicals were used directly without further purification.

2.2. Methods

2.2.1. Reaction of cisplatin with amino acids

Cisplatin and all amino acids were dissolved in ultrapure water to prepare the stock solutions of 4 mM cisplatin solution and 40 mM amino acid solutions, respectively. In order to fully dissolve Cys, Phe, Tyr and Trp amino acids, a small amount of formic acid was added. 200 μM cisplatin was incubated with each amino acid in ultrapure water at molar ratios of 1:1, 1:5 and 1:10, respectively and in 5 mM NH_4OAc buffer solutions at pH 3, 7, and 10 adjusted by adding HCOOH or NH_4OH at molar ratio of 1:5, respectively. These samples were sealed with parafilm and incubated in the dark at 37 °C for different times. Then they were diluted to 20 μM (cisplatin) with water immediately for MS analysis. As a control, 200 μM free cisplatin was hydrolyzed under the same condition. Cisplatin, a moderately toxic compound, should be handled carefully with protective equipment under safe environment including PVC gloves, lab coats and safety glasses, and the material safety data sheets should be known very well [23].

2.2.2. Electrospray ionization linear ion trap mass spectrometry (ESI LTQ MS)

MS^n experiments for the mixtures of cisplatin with amino acids were carried out on a LTQ XL linear ion trap mass spectrometer (Thermo, San Jose, CA, USA), which equipped with a conventional electrospray ionization source (ESI). The sample solutions were introduced into ESI MS by a 250 μL gas-tight syringe (Hamilton, Las Vegas) at a flow rate of 5 μL min^{-1} directly. The sheath gas was set at 30 (arbitrary units). The capillary temperature was set as 250 °C. The capillary voltage and tube lens voltage were set as 25 V and 110 V, respectively. The spray voltage was 5 kV. The zoom MS was used to obtain accurate *m*/*z* values of the complexes of cisplatin with amino acids. For the collision-induced dissociation (CID) experiments, nitrogen was used as collision gas, and 20–45% normalized collision energy defined by Thermo was used to fragment the platinated-amino acid ions. The *m*/*z* range of the isolated precursor ion peak was ± 0.5 Th. All samples were determined in the positive ion mode.

3. Results and discussion

3.1. Determination of the complexes of cisplatin with amino acids

In our study, the interactions of cisplatin with twenty kinds of essential amino acids were investigated in water and 5 mM NH_4OAc buffer solutions at pH 3, 7, and 10 respectively. Among them, water was used as the reaction medium to rule out the influences of organic solvents to the interactions of cisplatin and amino acids. The multiple platinated adducts with amino acids were recognized based on their accurate *m*/*z* values and the characteristic isotopic patterns using zoom MS.

3.1.1. Reactions of cisplatin with Met and Cys in different solutions

The reactions of cisplatin and Met in water at the molar ratios of 1:1, 1:5 and 1:10 over the time were determined. The similar reaction pathways occurred and reaction rate increased with increment of the molar ratios of Met and cisplatin. Here the reaction at the molar ratio 1:10 of cisplatin and Met was taken as an example and was shown in Fig. 1. Fig. 1a was mass spectrum of reaction mixture of Met with cisplatin in water at the molar ratio of 1:10 for 2 h. Ions including $[Pt(NH_3)_3Cl]^+$ and $[Pt(NH_3)_2Cl(H_2O)]^+$ from the hydrolysis of cisplatin were observed at low relative abundances, which were consistent with our previous study [24]. Five mono-adducts and one di-adduct were assigned as $[Pt(NH_3)_2Cl(Met)]^+$, $[Pt(NH_3)Cl(Met)]^+$, $[Pt(NH_3)_2(Met)-H]^+$, $[Pt(NH_3)(Met)+(HCOOH)-H]^+$, $[Pt(NH_3)(Met)-H]^+$ and $[Pt(Met)_2-H]^+$. The $[Pt(NH_3)(Met)+(HCOOH)-H]^+$ ion came from the ion reaction of $[Pt(NH_3)(Met)-H]^+$ with solvent HCOOH in gas phase during the electrospray ionization. Among them, the $[Pt(NH_3)Cl(Met)]^+$ with the highest abundance was the primary adduct, which suggested that the reaction process of Met with cisplatin was influenced seriously by the trans effect of sulfur (S) atom on Met. When the reaction time extended to 6 h, the peak of $[Pt(NH_3)_2Cl(Met)]^+$, $[Pt(NH_3)(Met)+(HCOOH)-H]^+$ and $[Pt(NH_3)_2(Met)-H]^+$ decreased and di-adduct $[Pt(Met)_2-H]^+$ was the highest abundance. Besides, $[Pt(NH_3)Cl(Met)]^+$ still was the predominant mono-adduct. As the reaction proceeded, the mono-adducts disappeared gradually, and only di-adduct $[Pt(Met)_2-H]^+$ was observed (Fig. 1b).

The reactions of cisplatin and Met at the molar ratio of 1:5 in NH_4OAc buffer solutions at pH 3, 7 and 10 over the time were determined. Supplementary Fig. S1 in the online version at DOI: http://dx.doi.org/10.1016/j.ijms.2016.09.017, showed the mass spectra of the reaction of cisplatin with Met in NH_4OAc buffer solution at pH 7 for different times. The platinated adducts