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Nuclease Digestion and Mass Spectrometric Characterization of Oligodeoxyribonucleotides Containing 1,2-GpG, 1,2-ApG, and 1,3-GpXpG Cisplatin Intrastrand Cross-links

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Abstract

Background—The primary mode of action for *cis*-diamminedichloroplatinum (II), referred to as cisplatin, towards the treatment of solid malignancies is through formation of cross-links with DNA at purine sites, especially guanines.

Methods—We prepared oligodeoxyribonucleotides (ODNs) containing a 1,2-GpG, 1,2-ApG, or 1,3-GpXpG cisplatin intrastrand cross-link and the corresponding ODNs modified with ¹⁵N₂-labeled cisplatin, and characterized these ODNs with electrospray ionization mass spectrometry (ESI-MS) and tandem MS (MS/MS). We also employed LC-MS/MS to characterize the digestion products of these ODNs after treatment with a cocktail of 4 enzymes (nuclease P1, phosphodiesterases I and II, and alkaline phosphatase).

Results—1,2-GpG was released from the ODNs as a dinucleoside monophosphate or a dinucleotide. Analyses of the digestion products of ODNs containing a 1,2-GpG cross-link on the 5' or 3' terminus revealed that the dinucleotide carries a terminal 5' phosphate. On the other hand, digestion of the 1,3-GpXpG intrastrand cross-link yielded 3 dinucleoside products with 0, 1, or 2 phosphate groups.

Results—The availability of the ODNs carrying the stable isotope-labeled lesions, MS/MS analyses of the cisplatin-modified ODNs, and the characterization of the enzymatic digestion products of these ODNs set the stage for the future LC-MS/MS quantification of the 1,2-GpG, 1,2-ApG, and 1,3-GpXpG lesions in cellular DNA.

Keywords

mass spectrometry; HPLC; stable isotope labeling; DNA digestion; and cisplatin

Introduction

Cancer chemotherapy was revolutionized with the serendipitous discovery of the antitumor properties of *cis*-diamminedichloroplatinum (II) (cisplatin, DDP). Since its approval for clinical use by the Food and Drug Administration in 1978, DDP is the most effective, and therefore, widely used therapeutic agent against epithelial malignancies including lung, neck, ovarian, bladder, and testicular cancers [1]. Cisplatin exerts its anticancer effects by

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inhibiting transcription, as well as targeting proteins and phospholipids; however, the most prominent mode of action involves the generation of DNA adducts which can inhibit DNA replication, and ultimately lead to cell cycle arrest and programmed cell death [1,2].

Cisplatin is intravenously administered in its neutral (inactive) form, $[\text{Pt}(\text{NH}_3)_2\text{Cl}_2]$. The concentration of chloride ions $[\text{Cl}^-]$ within the bloodstream (~ 100 mmol/l) is sufficiently high that the drug remains neutral and is able, by and large, to passively diffuse through the cell membrane [3]. Within the cell the $[\text{Cl}^-]$ decreases to 4–12 mmol/l, thus shifting the equilibrium towards aquation of DDP [3]. Water displaces the chloride ions to yield the mono-aqua-, $[\text{Pt}(\text{NH}_3)_2(\text{H}_2\text{O})\text{Cl}]^+$, and diaqua-, $[\text{Pt}(\text{NH}_3)_2(\text{H}_2\text{O})_2]^{2+}$, intermediate complexes, otherwise referred to as the active forms of cisplatin [3]. Both the mono-aqua- and diaqua-complexes have a strong kinetic preference for binding with DNA at the N7 position of guanine to form DNA cross-link lesions [4–6]. Specifically, activated cisplatin forms stable intrastrand cross-links between a guanine and: (1) an adjacent guanine (1,2-GpG); (2) a non-adjacent guanine separated by one nucleobase (1,3-GpXpG, where X = C or T); or (3) a 5'-neighboring adenine (1,2-ApG), at frequencies of 65%, 5–10%, and 25%, respectively [7]. An interstrand cross-link can also form between complementary strands of DNA on guanines at 5'-GpC/GpC-3' sites, as well as mono-adducts at the N7 position of the purine bases; however, these represent <1% of all cisplatin-DNA adducts [7].

The current challenges to cisplatin therapy are to minimize the side effects (i.e., nausea, vomiting, ototoxicity, and nephrotoxicity) of the drug while maintaining its potency against cancer cells, to extend successful treatment to a wider range of human cancers, and to determine the molecular mechanisms underlying drug resistance [8]. In order to address these challenges, it is necessary to have an analytical method that can simultaneously quantify all major cisplatin adducts in DNA. Given its inherent selectivity and sensitivity, liquid chromatography-tandem mass spectrometry (LC-MS/MS) is well suited to meet this need. LC-MS/MS was employed successfully for the quantification of the 1,2-GpG adduct using a stable isotope-labeled internal standard at a detection limit of 3 fmol/ μg DNA [9]; however, the described protocol was limited in that it could not be extended to include other cisplatin-DNA adducts. Moreover, the use of a single internal standard did not correct for the variation in enzymatic digestion efficiency that occurs due to the blocking nature of the different cisplatin adducts [10–22]. Thus, development of a quantitative LC-MS/MS-based method for the detection of cisplatin-bearing DNA necessitates optimization of the enzymatic digestion procedures for the release of the aforementioned cross-link lesions from DNA.

In the present study, we prepared oligodeoxyribonucleotides (ODNs) containing $^{15}\text{N}_2$ -labeled cisplatin-modified 1,2-GpG, 1,2-ApG, and 1,3-GpXpG adducts, as well as provided detailed MS/MS characterizations for both the light and heavy cisplatin-containing ODNs. We also analyzed the enzymatic digestion products formed from 1,2-GpG, 1,2-ApG, and 1,3-GpXpG cisplatin-containing ODNs using a 4-enzyme cocktail for digestion and LC-MS/MS for monitoring the digestion products. Our results led to the identification of two types of digestion products for the 1,2-GpG- and 1,2-ApG-bearing sequences, and three for the 1,3-GpXpG sequence.

Materials and Methods

Chemicals

Cisplatin, nuclease P1, snake venom phosphodiesterase (exoPI), calf spleen phosphodiesterase (exoPII), calf intestinal alkaline phosphatase (CIP), and 3-(6-aminopurin-9-yl)nonan-2-ol hydrochloride (EHNA) were from Sigma-Aldrich (St. Louis, MO). ^{15}N -labeled ammonium acetate was from Cambridge Isotope Laboratories, Inc.

(Andover, MA). Unmodified ODNs were from Integrated DNA Technologies (San Diego, CA).

Preparation of cisplatin-modified ODNs

Cisplatin-modified ODNs were prepared according to previously published protocols with modifications [21]. To activate cisplatin (conversion of *cis*-[Pt(NH₃)₂Cl₂] to *cis*-[Pt(H₂O)(NH₃)₂Cl]⁺ or *cis*-[Pt(H₂O)₂(NH₃)₂]²⁺), 4.5 mg cisplatin (15 nmol/μl) was treated with 12 μl of AgNO₃ stock solution (2.5 μmol/μL) and diluted to 1 ml with Millipore water. The samples were allowed to shake vigorously in the dark at 37°C for 24 hrs. The silver chloride precipitate was removed by centrifugation and the activated cisplatin (supernatant) was recovered. The single-stranded ODN sequences 1–5 (100 nmol) were treated with activated cisplatin (300 nmol, 20 μl) in a 1-ml reaction mixture containing 8.4 mmol/l sodium perchlorate. The reaction mixture was allowed to shake vigorously in the dark at 37°C for 4–5 h. The samples were concentrated by Speed-vac before immediate HPLC purification. The ¹⁵N₂-labeled cisplatin, [Pt(¹⁵NH₃)₂Cl₂], was synthesized following published protocols [23]. After recrystallization, we collected 282 mg of [Pt(¹⁵NH₃)₂Cl₂] at a yield of 33%. The isotope purity was confirmed using the higher resolution “zoom scan” ESI-MS analyses of treated ODN as described in the following sections. [Pt(¹⁵NH₃)₂Cl₂] was activated with AgNO₃ before treating the ODN sequences as described above for the unlabeled cisplatin.

Enzymatic Digestion

To 100 μg (~35 nmol) of dried, purified ODN samples (1–3) were added 0.05 or 0.2 units/μg DNA of nuclease P1, 6.3×10⁻⁵ or 2.5×10⁻⁴ units/μg DNA of calf spleen phosphodiesterase in a 20-μL buffer containing 300 mmol/l sodium acetate, 10 mmol/l Zn²⁺ (pH 5.0) and water to make the final volume of the solution 150 μl. The quantity of enzymes used represents ½ and 2-times the number of units required per μg of DNA based on vender's specifications, respectively. The digestion was carried out at 37°C for 6 h, and the enzymes were deactivated by heating to 95°C for 5 min. The resulting solution was dried by Speed-vac, and to the dried residues were added a 150-μL solution containing 40 μl of a 50 mmol/l Tris-HCl buffer (pH 8.6), 0.1 units/μg DNA of calf intestinal phosphatase, 2.5×10⁻⁴ units/μg DNA of snake venom phosphodiesterase, and water. EHNA was also added to the samples (1 nmol EHNA/4 μg DNA) to prevent deamination of 2'-deoxyadenosine to 2'-deoxyinosine (dI), which is important for off-line HPLC analysis because dI and the d(pGpG) digestion product coelute under the conditions used. The digestion mixture was incubated at 37°C for 4 h before an equal volume of chloroform was added to remove the enzymes. The pH of the aqueous layer was adjusted to neutral or slightly acidic conditions with 5% formic acid, and the aqueous layer was dried and redissolved in water prior to LC-MS/MS and HPLC analysis. Additionally, to 20 μg (~13 nmol) of dried, purified, cisplatinated ODN sequences 4 and 5 were added 2 units NP1, 0.003 units exoPII, 2 units CIP, and 0.005 units exoPI using similar procedures as described above.

HPLC

All cisplatin-modified ODNs were purified by reversed-phase HPLC using a Waters 2695 Separation Module (Milford, MA) coupled to a LinearTM UVIS 200 detector (set at 260 nm) and a Grace Apollo C18 column (4.6×250 mm, 5 μm in particle size, Part # 36511). A gradient of 10% B for 9 min (desalting), 10–20% B for 30 min, 20–75% B for 3 min, and 75% B for 6 min at a flow rate of 0.7 ml/min was employed, where mobile phase A contained 0.1 M triethylammonium acetate (TEAA) in water and mobile phase B contained 0.1 M TEAA in 80% acetonitrile. In most cases, a second purification was conducted to isolate the desired singly platinated ODN from the unmodified and multiply platinated ODNs. In this case, a gradient of 15% B for 9 min (desalting), 15–20% B for 30 min, 20–

75% B for 3 min, and 75% B for 6 min at a flow rate of 0.7 ml/min was used. All purified samples were dried by Speed-vac, redissolved in water for ESI-MS and MS/MS analyses, or for enzymatic digestion followed by LC-MS/MS analysis.

Off-line analysis of the digestion mixture was performed on an HPLC system as described above. A gradient of 5–15% B for 5 min, 15–30% B for 60 min, 30–98% B for 2 min, and 98% B for 10 min at a flow rate of 400 μ L/min was used, where buffer A contained 10 mmol/l ammonium formate in water and buffer B contained 10 mmol/l ammonium formate in 80% acetonitrile.

Mass Spectrometry

The ESI-MS and MS/MS analyses of cisplatin-modified ODNs were conducted on an LCQ Deca XP ion-trap mass spectrometer (ThermoFinnigan, San Jose, CA). An equal-volume solvent mixture of methanol and water was used as the carrier and electrospray solvent. A 2–5 μ l aliquot of each purified HPLC fraction was injected for sequence verification. The spray voltage was 3.6 kV and the mass width for precursor ion selection in MS/MS mode was 3 m/z units. Each spectrum was obtained by averaging 20–40 scans, and the time for each scan was 0.1 s.

LC-MS/MS

A Zorbax SB-C18 column (0.5×150 mm, 5 μ m in particle size, Agilent Technologies, Palo Alto, CA) and an Agilent 1200 Series capillary HPLC pump were employed for all LC-MS/MS experiments. A gradient of 5–10% B (5 min) and 10–35% B (40 min) was used at a flow rate of 6 μ L/min, where the mobile phases were 20 mmol/l ammonium acetate in water (A) and 20 mmol/l ammonium acetate in 40% acetonitrile (B). The effluent from the capillary column was coupled to an LTQ linear ion-trap mass spectrometer (Thermo Electron, San Jose, CA) operated in positive-ion mode in order to identify the cisplatin-containing products released upon enzymatic digestion.

Results and Discussion

Formation of cisplatin intrastrand cross-links

We begin by verifying the formation of the 1,2-GpG, 1,2-ApG, and 1,3-GpXpG intrastrand cross-links in the 10-mer ODNs that were treated with activated cisplatin. To this end, we subjected sequences 1, 2, and 3 (Table 1) to MS and MS/MS analyses. The negative-ion ESI-MS for the ODNs modified by unlabeled (light) cisplatin $[\text{Pt}(\text{NH}_3)_2]^{2+}$ (Fig. 1a; Supplementary Figures S2a and S4a) reflects a mass increase of 227 Da, relative to the unmodified ODN, which corresponds to the monoisotopic mass of the $[\text{Pt}(\text{NH}_3)_2]^{2+}$ less two protons. Similarly, the MS for the ODN treated with labeled (heavy) cisplatin $[\text{Pt}(^{15}\text{NH}_3)_2]^{2+}$ (Fig. 1c; Supplementary Fig. S2c and S4c) shows a mass increase of 229 Da, which is consistent with the monoisotopic mass of $[\text{Pt}(^{15}\text{NH}_3)_2]^{2+}$ less 2 protons. To further demonstrate the presence of a cisplatin adduct and to unambiguously distinguish between the light and heavy sequences, we also acquired higher-resolution “zoom-scan” MS to reveal the experimental isotopic distribution of the deprotonated ions of the platinated ODNs (Fig. 1b and 1d; Supplementary Fig. S2b, S2d, S4b, and S4d). For all sequences, the experimental isotopic distributions are in excellent agreement with the theoretical (calculated) ones. Therefore, we are confident that sequences 1, 2, and 3 were modified by the addition of one cisplatin adduct.

MS/MS analysis was implemented to confirm the location of the cisplatin intrastrand cross-link within the ODN sequences. Fragmentation of unmodified ODN by collision-induced dissociation (CID) typically gives rise to the a-B/w fragment ion pairs [24]. However, the

coordination of $[\text{Pt}(\text{NH}_3)_2]^{2+}$ or $[\text{Pt}(^{15}\text{NH}_3)_2]^{2+}$ to the ODN resulted in unique fragment ions, as illustrated in Figures 3, 5, and 7. Beginning with the 1,2-GpG-containing ODN (sequence 1, Fig. 3), the $a_5\text{-G}/w_5^*+\text{G}$ ion pair is readily detectable, which arises from the release of the 5th nucleobase (counting from the 5' end) from the backbone of the ODN, and cisplatin (*) coordination to the 5th and 6th guanine residues, which are retained on the fragment ion carrying the 3' terminus (i.e., w_5^* ion). Additionally, the $a_5\text{-G}/w_5^*$ ion pair was identified, which reflects the loss of the 5th guanine and retention of only cisplatin on the 6th nucleobase. To a lesser degree, we found the a_5^*/w_5 fragment-ion pair in which the cisplatin is coordinated with the 5th guanine base that remains bound to the deoxyribose on the 5' fragment ion.

We also observed unique cleavage between the 6th and 7th nucleotides to yield the $a_6^*\text{-G}/w_4$ and a_6^*/w_4 fragment ion pairs. The $a_6^*\text{-G}$ and a_6^* ions form from the loss of the 6th nucleobase with cisplatin remaining on the 5th guanine, and retention of both the 6th guanine, albeit cleaved from the deoxyribose, and cisplatin on the fragment ion carrying the 5' terminus, respectively. Ions arising from the loss of one, and in many cases, both ammine groups for the light and heavy cisplatin-containing fragments were also identified. The presence of fragment ions with cisplatin bonded with the 5th or 6th guanine, or both simultaneously, may be explained by the metal complex nature of the adduct coordination to the N7 position of guanine, as opposed to covalent bonds, which allows for mobility of platinum moiety between the two nucleobases. Based on the ions generated upon cleavage at the $a_5\text{-B}/w_5$ and $a_6\text{-B}/w_4$ sites, coupled with a previously published report describing a comparable fragmentation pattern for cisplatin-harboring ODN with a 1,2-GpG motif [25], it is evident that the $[\text{Pt}(\text{NH}_3)_2]^{2+}$ and $[\text{Pt}(^{15}\text{NH}_3)_2]^{2+}$ adducts are complexed with both the 5th and 6th guanines, thus confirming the presence of a bifunctional intrastrand cross-link.

Although analogous in trend, to our knowledge we are the first to fully characterize the fragmentation pattern of ODNs carrying a 1,2-ApG (sequence 2, Figure 5) or 1,3-GpXpG (sequence 3, Fig. 7) cisplatin intrastrand cross-link. For sequence 2, we observed the $a_5\text{-A}/w_5^*+\text{A}$ ion pair, which arises from the cleavage of *N*-glycosidic bond of the 5th nucleoside, and the retention of the 5th nucleobase (i.e., adenine) along with the cisplatin on the 6th guanine of the 3' fragment ion. The $a_5\text{-A}/w_5^*$ and a_5^*/w_5 fragments are also present, indicating the loss of the 5th adenine with only the cisplatin remaining on the 6th guanine, and the retention of the 5th adenine as well as cisplatin on the 5'-fragment ion, respectively. As with the 1,2-GpG-containing sequence, the a_5^* fragment is significantly less abundant than $w_5^*+\text{A}$ and w_5^* .

In the case of sequence 3, we again identified ion pairs where the cisplatin adduct was present on the 5' fragment ions (a_4^*/w_6 and a_6^*/w_4), or 3' fragment ions either with ($a_4\text{-G}/w_6^*+\text{G}$) or without ($a_4\text{-G}/w_6^*$) the guanine being cleaved from the 5' deoxyribose. As was observed with sequences 1 and 2, ions representing the loss of one or both of the cisplatin amines were abundantly present in the product-ion spectra for sequence 3, which was described in part previously [25,26]. Complete assignment of the MS/MS for all sequences may be found in Figures 2, 4, and 6 and Supplementary Figures S1, S3, and S5. Additionally, we verified the coordination of one cisplatin adduct to the 5-mer sequences (4 and 5) by MS and zoom-scan MS (Supplementary Fig. S6).

Identification of Enzymatic Digestion Products

We next digested the aforementioned cisplatin-modified ODNs with a 4-enzyme cocktail of NP1, exoPII, CIP, and exoPI and analyzed the enzymatic digestion products using LC-MS/MS. We chose these four enzymes because they have been employed successfully for the efficient release of other intrastrand cross-links from isolated and cellular DNA [27–30]. Moreover, this combination of enzymes has not been previously reported for digesting

cisplatin-modified DNA, though its usefulness towards developing a quantitative assay of $\text{Pt}(\text{NH}_3)_2$ -bearing DNA was suggested [22].

Figure 8 displays the products housing the cisplatin remnant that we identified from LC-MS/MS analysis (Supplementary Fig. S7-S13). As was previously reported with unlabeled cisplatin, we observed m/z values corresponding to cisplatin-chelated (*) 1,2-GpG dinucleoside digestion products harboring one [structure II, $\text{d}(\text{G}^*\text{pG}^*)$] [9,11,12,16,17,20,22,31] or two [structure I, $\text{d}(\text{pG}^*\text{pG}^*)$ or $\text{d}(\text{G}^*\text{pG}^*\text{p})$] [13,15,18,19,22,31–35] phosphate groups. Similarly, a cisplatin-containing 1,2-ApG dinucleoside digestion products bearing one [structure IV, $\text{d}(\text{A}^*\text{pG}^*)$] or two [structure III, $\text{d}(\text{pA}^*\text{pG}^*)$ or $\text{d}(\text{A}^*\text{pG}^*\text{p})$] [11–13,15,17–20] phosphate groups were observed. The digestion products for the non-adjacent 1,3-GpXpG-harboring motif differed from the adjacent cross-links in that the phosphodiester bonds located in between the cisplatin adduct were cleaved. This gave rise to dinucleoside digestion products with zero [structure VII, $\text{d}(\text{G}^*\text{G}^*)$] [11,12,20], one [structure VI, $\text{d}(\text{pG}^*\text{G}^*)$ or $\text{d}(\text{G}^*\text{G}^*\text{p})$], or two [structure V, $(\text{dG}^*\text{MP})_2$] [13,18,19] attached phosphate groups, where, to our knowledge, structure VI has not been previously reported. The accessibility of the phosphodiester bonds in the non-adjacent intrastrand cross-link may be explained by its distortion of DNA. NMR [36] and molecular dynamic studies [37] have shown that the central nucleotide ($X = \text{T}$ [36] or C [37]) is looped out away from the other nucleotides, thus exposing the phosphodiester bonds and rendering them available for nuclease cleavage. In the 1,2-GpG and 1,2-ApG cross-links the interposed phosphodiester bonds do not protrude in this way.

Structure Verification of Dinucleotide Digestion Products

Although structures I and III were previously reported, we found some ambiguity in the literature regarding to whether the terminal phosphate resides on the 5' or 3' end of the dinucleotide [15,22,31]. To address this question directly, we synthesized cisplatin-bearing 5-mers in which the GG motif was located on either the 3'-(sequence 4) or 5'-(sequence 5) terminus, and then subjected both to enzymatic hydrolysis. The HPLC trace of the digestion mixture for each sequence is provided in Figures 9a and 9b. For sequence 4, the peak at ~ 25 min represents the major digestion product, $\text{d}(\text{pG}^*\text{pG}^*)$, in which the phosphate resides on the 5'-side of the cisplatin adduct. A trace amount of the complete digestion product, $\text{d}(\text{G}^*\text{pG}^*)$, was also detected at ~ 46 min. Conversely, sequence 5 yielded only the $\text{d}(\text{G}^*\text{pG}^*)$ dinucleoside at ~ 46 min. Together, these findings unequivocally demonstrate that the terminal phosphate observed in structure I resides solely on the 5'-side of the cisplatin adduct. The nature of both digestion products were confirmed by MS analysis (Figure 9c and 9d). Given that the 1,2-ApG and 1,2-GpG intrastrand cross-links form in the same manner [4,38,39], we may deduce that the terminal phosphate for structure III is also located on the 5'-end.

Conclusions

Cisplatin is the most widely used chemotherapeutic agent for the treatment of epithelial malignancies. However, its administration is met with challenges that include severe side effects, a limited breadth of effectiveness against certain common tumors, and an acquired or intrinsic drug resistance. To overcome these limitations, it is necessary to have a quantitative analytical method that can characterize the interactions between cisplatin and biomolecules, especially DNA. The selectivity and sensitivity of LC-MS/MS makes it an ideal tool for this task. In the present study, we prepared ODN substrates housing the light or heavy ($^{15}\text{N}_2$) cisplatin-modified 1,2-GpG, 1,2-ApG, or 1,3-GpXpG intrastrand cross-link, and characterized these modified ODNs with ESI-MS and MS/MS analyses. The MS/MS

results confirmed the sites of modification and also revealed that the cisplatin cross-link led to formation of unique fragment ions arising from cleavage at the modification sites.

We also digested the cisplatin-modified ODNs with a cocktail of four enzymes and characterized the digestion products using LC-MS/MS. Similar as previously reported [9,11–13,15–20,22,31–35], digestion of the 1,2-GpG and 1,2-ApG sequences gave rise to dinucleoside products carrying an internal phosphate (Fig. 8, structures I and III, respectively), or containing both an internal and a terminal phosphate (Fig. 8, structures II and IV, respectively). By analyzing digestion products of ODNs containing a 1,2-GpG on the 5' or 3' terminus, we demonstrated unambiguously that the terminal phosphate group was located on the 5'-side of the cisplatin adduct. Similar as what were described in the literature [11–13,18–20], we also detected the d(G*G*) and (dG*MP)₂ products (Fig. 8, structures V and VII, respectively) upon digestion of the 1,3-GpXpG cisplatin-containing DNA. Additionally, we reported a new digestion product (structure VI, Fig. 8) for the 1,3-GpXpG cross-link.

The formation of multiple digestion products for each of the 3 intrastrand cross-link products supports that the accurate quantification of these cisplatin DNA adducts requires the use of ODN substrates housing the stable isotope-labeled lesions. Thus, the availability of ODNs containing stable isotope-labeled standard 1,2-GpG, 1,2-ApG, and 1,3-GpXpG cisplatin-modified DNA adducts and the examination of the enzymatic digestion products facilitated the future LC-MS/MS quantification studies of these DNA lesions in cultured human cells or in patients undergoing cisplatin therapy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

cisplatin or DDP	<i>cis</i> -diamminedichloroplatinum (II)
ODN	oligodeoxyribonucleotide
ESI	electrospray ionization
CID	collisionally induced dissociation

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Highlight

- Oligodeoxyribonucleotides with unlabeled and $^{15}\text{N}_2$ -labeled cisplatin were prepared
- These modified DNA were characterized in detail by ESI-MS and MS/MS
- The enzymatic digestion products for the modified DNA were identified
- This study facilitates the future LC-MS/MS quantification of cisplatin DNA adducts

Figure 1.

Negative-ion ESI-MS (a,c) and high-resolution "zoom-scan" analysis showing the experimental isotopic distribution of the $[\text{M}-3\text{H}]^{3-}$ ion with an inset depicting the corresponding theoretical isotopic distribution (b,d) of unlabeled (a,b) and $^{15}\text{N}_2$ -labeled (c,d) d(ATCCG*G*CCTA), where * represents light $[\text{Pt}(\text{NH}_3)_2]^{2+}$ or heavy $[\text{Pt}(^{15}\text{NH}_3)_2]^{2+}$ cisplatin coordination, respectively.

Figure 2. Negative-ion ESI-MS/MS of the unlabeled $[M-3H]^{3-}$ ion with an inset showing a list of the observed fragment ions (a) and the peak assignments (with relative ion abundance in parenthesis) for the product-ion spectrum (b) of d(ATCCG*G*CCTA) where * (a) and bold m/z values (b) represent $[Pt(NH_3)_2]^{2+}$ coordination.

Figure 3.

Unique fragment ion pairs and the corresponding m/z values $\{[Pt(NH_3)_2]^{2+}/[Pt(^{15}NH_3)_2]^{2+}\}$ generated during the CID of ODN sequence 1, which harbors a 1,2-GpG cisplatin (*) intrastrand cross-link. Numbers in parenthesis represents relative ion abundance.

Figure 4. Negative-ion ESI-MS/MS of the unlabeled $[M-3H]^{3-}$ ion with an inset showing a list of the observed fragment ions (a) and the peak assignments (with relative ion abundance in parenthesis) for the product-ion spectrum (b) of d(ATCCA*G*CCTA) where * (a) and bold m/z values (b) represent $[Pt(NH_3)_2]^{2+}$ coordination.

Figure 5.

Unique fragment ion pairs and the corresponding m/z values $\{[Pt(NH_3)_2]^{2+}/[Pt(^{15}NH_3)_2]^{2+}\}$ generated during the CID of ODN sequence **2**, which harbors a 1,2-ApG cisplatin (*) intrastrand cross-link. Numbers in parenthesis represents relative ion abundance.

Figure 6. Negative-ion ESI-MS/MS of the unlabeled $[M-3H]^{3-}$ ion with an inset showing a list of the observed fragment ions (a) and the peak assignments (with relative ion abundance in parenthesis) for the product-ion spectrum (b) of d(ATCG*CG*CCTA) where * (a) and bold m/z values (b) represent $[Pt(NH_3)_2]^{2+}$ coordination.

Figure 7.

Unique fragment ion pairs and the corresponding m/z values $\{[Pt(NH_3)_2]^{2+}/[Pt(^{15}NH_3)_2]^{2+}\}$ generated during the CID of ODN sequence **3**, which harbors a 1,2-GpXpG cisplatin (*) intrastrand cross-link. Numbers in parenthesis represents relative ion abundance.

Figure 8.

Structures and m/z values for the $[M+H]^+$ ions for light $[Pt(NH_3)]^{2+}$ / heavy $[Pt(^{15}NH_3)]^{2+}$ cisplatin adducts of the enzymatic digestion products.

Figure 9. HPLC traces for the separation of the enzymatic digestion mixture of synthetic sequences **4** (a) and **5** (b) upon cisplatin treatment; and the corresponding positive-ion ESI-MS of the hydrolysis products d(pG*pG*) (c) and d(G*pG*) (d), where * indicates the coordination of a cisplatin adduct.

Table 1

The ODN sequences employed for enzymatic digestion experiments.

Sequence Number	Abbreviation	Sequences
1	1,2-GpG	5'-d(ATCCGGCCTA)-3'
2	1,2-ApG	5'-d(ATCCAGCCTA)-3'
3	1,3-GpXpG	5'-d(ATCGCGCCTA)-3'
4	3'-1,2-GpG	5'-d(TCTGG)-3'
5	5'-1,2-GpG	5'-d(GGTCT)-3'