Analysis of Platinum Adducts with DNA Nucleotides and Nucleosides by Capillary Electrophoresis Coupled to ESI-MS: Indications of Guanosine 5′-Monophosphate O^6–$N7$ Chelation

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DNA is the ultimate target of platinum-based anticancer therapy. Since the N7 of guanine is known to be the major binding site of cisplatin and its analogues, adduct formation with model nucleotides, especially 2′-deoxyguanosine 5′-monophosphate (dGMP), has been studied in detail. During the last few years a coupled capillary eletrophoresis/electrospray-ionization mass spectrometry (CE/ESI-MS) method has been advantageously used in order to separate and identify platinum adducts with nucleotides in submillimolar concentrations in aqueous solutions. Beside the bis-adduct, $[Pt(NH_3)_2(dNMP)_2]^{2-}$ (NMP = 2′-deoxynucleoside 5′-monophosphate), and the well-known monochloro and monohydroxo adducts, $[Pt(NH_3)_2Cl(dNMP)]^-$ and $[Pt(NH_3)_2(dNMP)OH]^-$, respectively, a third kind of monoadduct species with a composition of $[Pt(NH_3)_2(dNMP)]^-$ can be separated by CE and detected through the m/z values measured with ESI-MS. Different experimental setups indicate the existence of an O^6–N7 chelate, whereas the formation of N7–αPO_4 macrochelates or dinuclear species is unlikely. Additionally, offline MS experiments with 2′-deoxyguanosine (dG) and stabilization of the controversially discussed O^6–N7 chelate by oxidation with hydrogen peroxide support the assumption of the existence of O^6–N7 chelation.

Introduction

Cisplatin (*cis*-diamminedichloroplatinum(II), CDDP) is one of the most widely used antitumor drugs and is effectively applied in the therapy of testicular and ovarian cancer. It is generally accepted that the ultimate target of cisplatin and its analogues is DNA. The anticancer activity of these compounds is derived from their ability to cross-link the DNA nucleotides in cancer cells and thereby modify the conformation of the DNA.[1,2] The major platination site of cisplatin is known to be the *N7* atom of guanine; this predominantly results in 1,2-d(GpG) intrastrand cross-links (65%; p = linking phosphate group, dG = 2′-deoxyguanosine). 1,2-d(ApG) intrastrand, 1,3-d(GpNpG) intrastrand, and interstrand cross-links are formed to a minor extent (dA = 2′-deoxyadenosine, dN = 2′-deoxynucleoside).[3–8] Formation of intrastrand adducts was found to cause inhibition of DNA replication.[9] As a last consequence adduct formation leads to the induction of programmed cell death (apoptosis). DNA binding has been investigated in numerous studies[7,10,11] including research into the kinetics of cross-linking DNA with cisplatin.[11–15]

One theory, postulated when research in this field began, considered participation of the O^6 atom of the guanine residue in binding with cisplatin; this would result in a bidentate O^6–*N7* chelate complex (Scheme 1 a). Chelation of different metal centers by the *N7* atom and either the O^6 or the 6-NH_2 moiety of purine bases (guanine and adenine, respectively) has been suggested. The theory that an O^6–*N7* chelate is formed when guanosine is platinated was proposed several times and is still discussed, although it is controversial.[16–22] First results obtained from ^{1}H NMR spectroscopy indicated that an O^6–*N7* chelate is probably not formed.[23] On the other hand, formation of analogous O^6–*N7* chelate complexes has been observed for some related sulfur derivatives with palladium(II)[24] and in a Cu^{II} complex with 6-thio-9-methylpurine.[25] Additionally, in the case of Cu^{II}, a deprotonated theophylline ligand was found to coordinate through O^6 and *N7*.[26]

In organometallic platinum(IV) chemistry, the crystal structure of a trimethylplatinum(IV)–theophylline hexamer with O^6–

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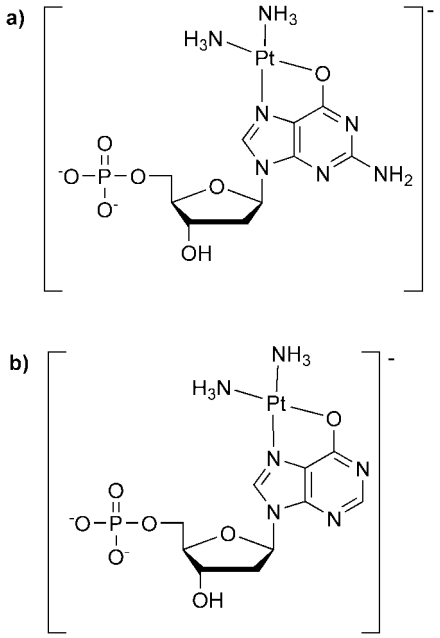
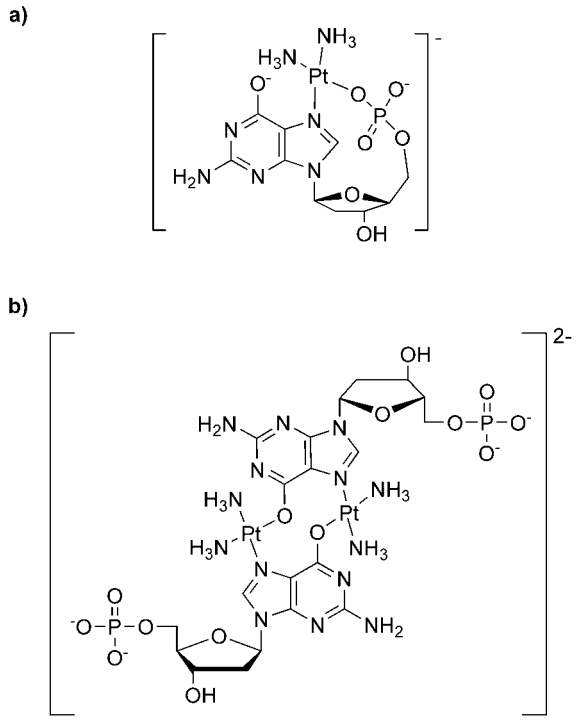


Scheme 1. *Structural formulae of the proposed O^6–N7 chelates for the reaction of cisplatin with 2′-deoxyguanosine 5′-monophosphate (dGMP; a) and 2′-deoxyinosine 5′-monophosphate (dIMP; b).*

*N*7 chelation has been reported.[27] ^{1}H and ^{31}P NMR spectroscopy supported by molecular mechanics revealed that, instead of the O^6–*N*7 bidentate coordination, *N*7–αPO_4 macrochelation might occur during the reaction of cisplatin and 2′-deoxyguanosine 5′-monophosphate (dGMP; Scheme 2 a).[28,29]



Scheme 2. *Structural formulae of the macrochelate complex formed between dGMP and cisplatin through* N7 *and αPO_4 (a) and a dimeric complex formed between two cisplatin and two dGMP units (b).*

However, to date no structural information has been obtained confirming the existence of a direct bond between the guanine O^6 atom and Pt^{II} coordination compounds when they are coordinated to the *N*7 atom of the guanine. Some recent investigations on the hydrolysis of platinum complexes and their binding behavior toward nucleotides demonstrated the suitability of capillary electrophoresis (CE) in this field of research. The possibility of simulating physiological conditions in the experimental setup is especially noteworthy.[30–37]

In order to investigate the impact of individual platinum–DNA adducts on antitumor activity, as well as the toxicity of platinum complexes used in cancer therapy, a method for the separation and identification of platinum adducts with nucleotides was developed that utilizes capillary electrophoresis coupled to electrospray-ionization mass spectrometry (ESI-MS).[34,38,39] This powerful analytical technique combines both the high separation efficiency of CE and the high selectivity of MS. Online coupling allows distinct investigations of the structure of analytes directly after separation in the capillary. The benefit of applying CE is the possibility of separating positively and negatively charged, as well as neutral species in aqueous media in one single run. Generally, in positive CE mode, all peaks found with migration times shorter than the electroosmotic flow (EOF) bear positive charges. Anions, in contrast, migrate slower than the EOF, and neutral compounds are found to comigrate with the EOF. Direct introduction of the end of the capillary into the ESI source allows detection in the gas phase of species present in the solution.[40,41]

Applying this method to the monitoring of the reaction of nucleotides with cisplatin, we observed well-separated individual platinum adducts. In this report, we present detailed investigations of adduct formation of 6-oxopurine nucleotides and nucleosides upon interaction with cisplatin by means of a coupled CE/ESI-MS method with different reaction conditions and ESI-MS in offline mode. The results suggest that for the interaction of platinum(II) complexes with DNA purine bases (and derivatives of them) O^6–*N*7 chelation is a possible coordination mode.

Results and Discussion

In previous work, the feasibility of CE equipped with a UV/Vis diode array detector[30–33,35,36] or coupled to an ESI-MS system for the separation and identification of cisplatin adducts with nucleotides has been demonstrated.[34,36–39] With respect to structure–activity relationships and in order to elucidate the mode of action of anticancer platinum complexes, CE can, due to high separation efficiency, advantageously be used a) for investigations in buffered aqueous solutions, even in the presence of high chloride concentrations, b) for the identification of platinum adducts with nucleotides at submillimolar concentrations (0.05 mM), and c) for the separation of platinum adducts due to their charge and radii.

The charge of the complexes under investigation depends on the conditions of the CE (pH 7.4) and MS (acidic sheath liquid). To give an example: Under CE conditions (buffered solution at pH 7.4 or higher) the monochloro adduct between

cisplatin and dGMP is a single negatively charged complex, whereas under MS conditions with an acidic sheath liquid the phosphate oxygen atoms become protonated with the result being a single positively charged complex.

Table 1. *Calculated m/z ratios of cisplatin–2′-deoxynucleotide adducts (corresponding to protonated analytes with respect to the acidic sheath liquid used).*

Nucleotide	Adduct type	Formula	*m/z*
dGMP	$[Pt(NH_3)_2(dGMP)]^+$ [a]	$[C_{10}H_{19}N_7O_7PPt]^+$	575
	$[Pt(NH_3)_2(dGMP)OH]^+$	$[C_{10}H_{21}N_7O_8PPt]^+$	593
	$[Pt(NH_3)_2Cl(dGMP)]^+$	$[C_{10}H_{20}ClN_7O_7PPt]^+$	611
	$[Pt(NH_3)_2(dGMP)_2]^+$	$[C_{20}H_{33}N_{12}O_{14}P_2Pt]^+$	922
dIMP	$[Pt(NH_3)_2(dIMP)]^+$ [a]	$[C_{10}H_{18}N_6O_7PPt]^+$	560
	$[Pt(NH_3)_2(dIMP)OH]^+$	$[C_{10}H_{20}N_6O_8PPt]^+$	578
	$[Pt(NH_3)_2Cl(dIMP)]^+$	$[C_{10}H_{19}ClN_6O_7PPt]^+$	596
	$Pt(NH_3)_2(dIMP)_2]^+$	$[C_{20}H_{31}N_{10}O_{14}P_2Pt]^+$	892

[a] O^6–*N7* chelate.

Results obtained with experimental setup A

CE/ESI-MS was applied to examine the reaction of dGMP with cisplatin. Incubation of cisplatin and dGMP at a molar ratio of 1:1 (0.5 mM) in water at 37 °C resulted in the formation of three different monoadducts and the bisadduct $[Pt(NH_3)_2(dGMP)_2]^+$ (*m/z*: 922; see Figure 1 for the single-ion electropherograms). Two of the monoadduct peaks could be assigned to the monochloro and the monohydroxo adducts, $[Pt(NH_3)_2Cl(dGMP)]^+$ and $[Pt(NH_3)_2(dGMP)OH]^+$, with *m/z* values of 611 and 593, respectively. These *m/z* values are in good agreement with the calculated ones (Table 1). Surprisingly, a third monoadduct peak migrating faster than all other platinum–dGMP adducts could be observed with an *m/z* value of 575; this is in accordance with a $[Pt(NH_3)_2(dGMP)]^+$ species (Figures 1 and 2).

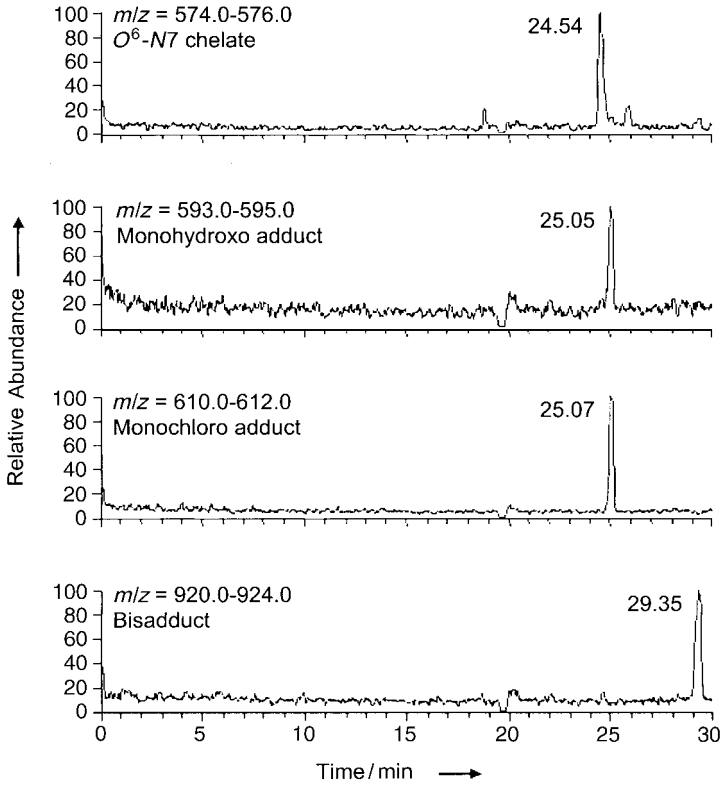


Figure 1. *Single-ion electropherograms of the individual adduct peaks. The chelate with an m/z value of 575 was formed in a solution of cisplatin and dGMP (molar ratio 1:1; Exp. A; 6 h incubation at 37 °C; see the Experimental Section).*

When dGMP was exchanged with the structurally similar nucleotide 2′-deoxyinosine 5′-monophosphate (dIMP, equivalent to dGMP without the 2-NH_2 group; Scheme 1b) and the same experimental setup was applied, a qualitatively comparable peak pattern was observed (data not shown). In migration order, peaks with *m/z* values of 560, 578, 596, and 892 were determined to represent a $[Pt(NH_3)_2(dIMP)]^+$ species, the monohydroxo, the monochloro, and the bisadduct $[Pt(NH_3)_2(dIMP)_2]^+$, respectively. In Figure 3 the full mass scan obtained for the peak migrating with the highest velocity, $[Pt(NH_3)_2(dIMP)]^+$, is displayed.

The following species could account for *m/z* values of 575 and 560 (corresponding to incubation with dGMP or dIMP):

1) a platinum O^6–*N7* chelate (Scheme 1)
2) an *N7*–αPO_4 macrochelate (Scheme 2a)
3) a doubly charged dinuclear species (Scheme 2b)

Although mass peaks at *m/z* values of 575 and 560 are assignable to the doubly charged dinuclear species $[Pt_2(NH_3)_4(dGMP)_4]^{2+}$ and $[Pt_2(NH_3)_4(dIMP)_4]^{2+}$, respectively, the existence of such Pt–dNMP adducts can be ruled out by taking into account the difference in the isotopic pattern of singly and doubly charged ions (NMP = 2′-deoxynucleoside 5′-mono-

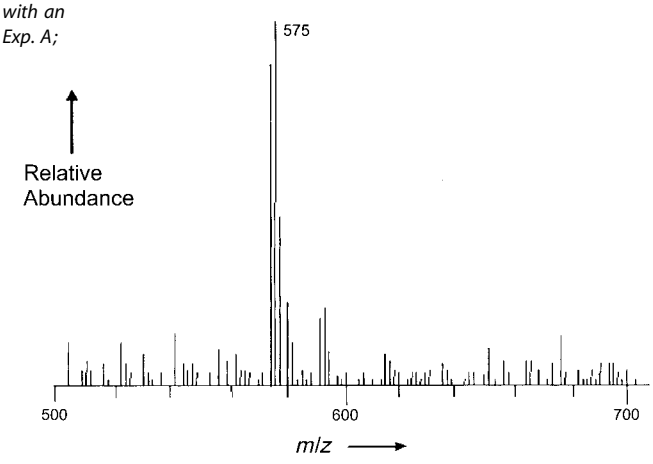


Figure 2. *Full-scan mass spectrum at 24.54 min. The mass track of the O^6–N7-dGMP–cisplatin species is shown in Figure 1 (Exp. A; 6 h incubation of dGMP with cisplatin; see the Experimental Section).*

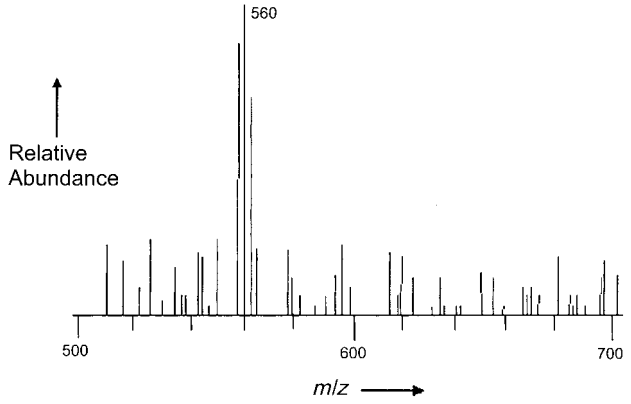


Figure 3. *Full-scan mass spectrum at 18.10 min, related to the mass track of the O^6–N7-dIMP–cisplatin species (Exp. A; 5 h incubation of dIMP with cisplatin; see the Experimental Section).*

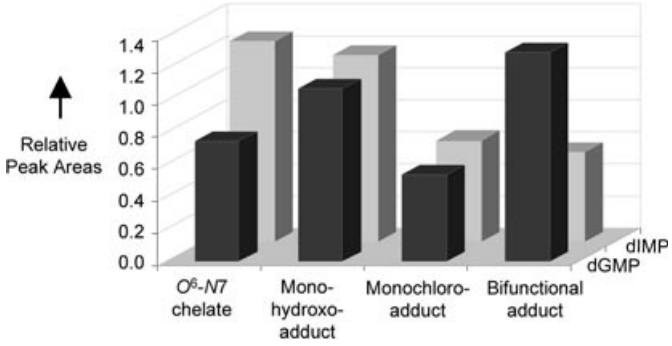


Figure 4. *Comparison of the relative peak areas between dGMP– and dIMP–platinum adducts (Exp. A; 5 h incubation; see the Experimental Section).*

phosphate). In fact, isotopic patterns with a mass-unit difference of 1 *m/z* are detected, a fact indicating the existence of singly charged species (compare Figures 2 and 3), while doubly charged ions would result in an isotopic distribution with $\Delta m/z = 0.5$ between the isotope peaks.

A *N7*–αPO_4 macrochelate, which has already been described,[28,29] can not be conclusively excluded by applying experimental setup A. Comparison of the product distribution of the incubation of cisplatin with either dGMP or dIMP (both at molar ratios of 1:1) gives another hint of the existence of the proposed O^6–*N7* chelate or at least of a coexistence of both the latter and the *N7*–αPO_4 macrochelate. Whereas the adducts $[Pt(NH_3)_2Cl(dNMP)]^+$ and $[Pt(NH_3)_2(dNMP)OH]^+$ of both nucleotides were formed to nearly the same extent, the relative peak area between the dGMP bisadduct, $[Pt(NH_3)_2(dGMP)_2]^+$, and the O^6–*N7* chelate shows a ratio of 2:1, while in the case of dIMP a reciprocal ratio was found (Figure 4). This striking difference is not explainable by macrochelate formation since, due to the coordination sphere at the platinum(II) center, there should be no remarkable preference for a *N7*–αPO_4 species if either dGMP or dIMP are used. These significant deviations in product distribution are rather explainable by a marked difference in the pK_a values of the *N1* atom of the purine bases. The proton at the *N1* position of dIMP is found to be more acidic than the *N1*(H) atom of dGMP (9.02 versus 9.49).[42] Moreover, it is known that after coordination of platinum(II) to the *N7* atom the pK_a values of the *N1* protons are additionally decreased by nearly one unit.[43] This change in acidity of the *N1*(H) proton may be the driving force for the formation of the O^6–*N7* chelate, especially in the case of dIMP.

Results obtained with experimental setup B

In order to support the hypothesis of O^6–*N7* chelate formation, experiments have also been carried out under more physiological conditions in buffered solution at pH 7.4. CE/ESI-MS and offline-MS experiments resulted in a similar peak pattern and equivalent *m/z* values. In the case of a cisplatin-to-dGMP incubation ratio of 1:1, signals in the mass spectra were observed that are in accordance with the calculated *m/z* ratio for the O^6–*N7* chelation (Figures 5 and 6). The O^6–*N7* chelate peak could be detected even in samples incubated at a cisplatin-to-

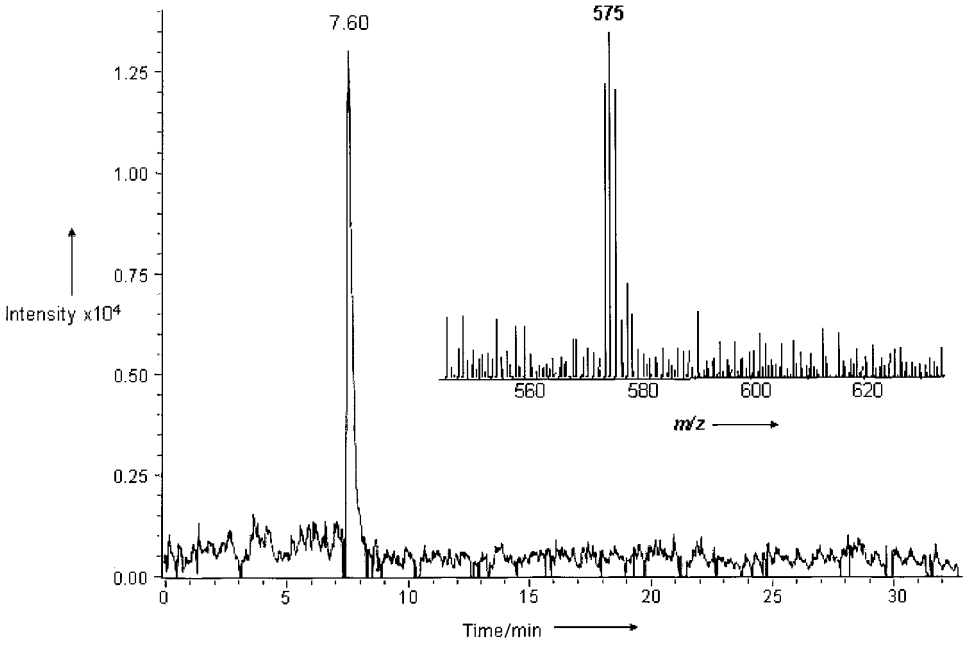


Figure 5. *Single-ion electropherogram and mass spectrum at 7.6 min of the chelate with an m/z value of 575 formed in a buffered solution (pH 7.4) containing cisplatin and dGMP (molar ratio 1:1; Exp. B; 24 h incubation at 37°C; see the Experimental Section).*

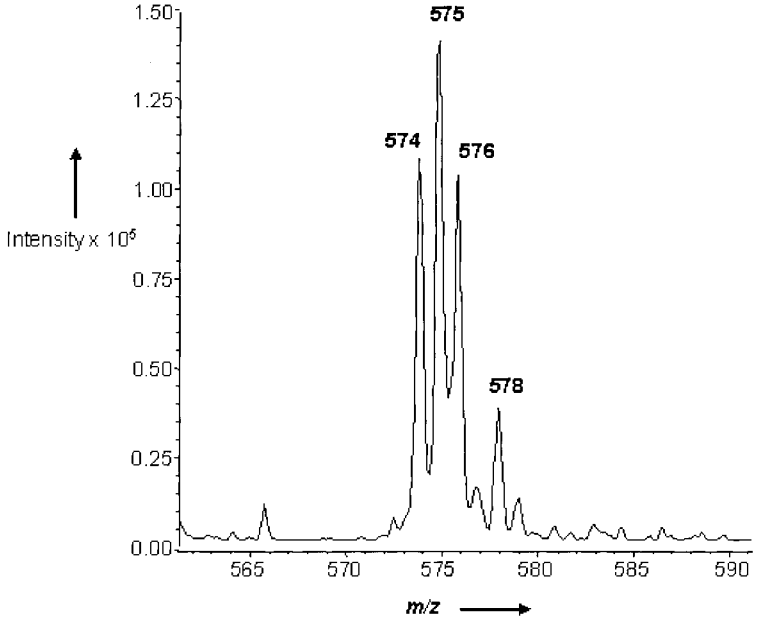


Figure 6. *Offline mass spectrum of cisplatin incubated with dGMP (molar ratio 1:1, 1 mM) for 7 h at pH 7.4 and 37 °C (Exp. B; see the Experimental Section).*

dGMP ratio of 1:2. Furthermore, experiments have shown that the chelate seems to be rather stable once it is formed, since subsequent addition (after incubation for 24 h of cisplatin with dGMP at a molar ratio of 1:1) of a twofold excess of dGMP does not substantially shift the equilibrium towards the formation of the bisadduct.

With the intention of definitely excluding the presence of the *N*7–αPO_4 macrochelate species, cisplatin was also incubated with 2′-deoxyguanosine under physiological conditions (pH 7.4). Since adduct formation with dG results in a series of neutral species that cannot be separated with the applied experimental setups, the measurements were performed by using offline ESI-MS (see Table 2 for the expected *m/z* values of the adducts). Actually, the *m/z* value of 495 for the postulated O^6–*N*7 chelate could also be detected in case of incubation with dG (Figure 7). In comparison with the intensities obtained for the chelate peaks after incubation of cisplatin with dGMP or dG, the chelate peak assigned to $[Pt(NH_3)_2(O^6\text{–}N7\text{-dG})]^+$ (*m/z* 495) was found to have lower intensity. Consequently, for the reaction between cisplatin and dGMP, coexistence of the O^6–*N*7 chelate and the *N*7–αPO_4 macrochelate is conceivable and explains the differences in peak intensities.

Since platinum(IV) complexes are known to be more stable and kinetically inert and since an analogous O^6–*N*7 chelation has been reported for a trimethylplatinum(IV)-theophylline hexamer, we tried to trap the O^6–*N*7 chelate as its platinum(IV) species by oxidation with hydrogen peroxide.[20] In order to oxidize Pt^{II} to Pt^{IV}, H_2O_2 (30%, 2.3 mM) was added to the cisplatin–dG reaction mixture after 24 h of incubation (1 mM, 1:1, in 20 mM ammonium acetate buffer at pH 7.4 and 37 °C). To ensure that the suggested O^6–*N*7 chelate was formed, ESI-MS measurements of the solution were performed before addition of hydrogen peroxide and resulted in a comparable full-scan spectrum to that described before (see Table 2). After addition of H_2O_2, the solution was kept over night at 37 °C. Afterwards, ESI-MS experiments were again performed. Beside several oxidation products, mass-to-charge ratios for the oxidized bisadduct and the platinum(IV) monoadducts could be observed as being in good agreement to the calculated ratios (see Table 3). A peak with an *m/z* value of 529 strongly indicates the existence of the dihydroxoplatinum(IV)–O^6–*N*7-dG chelate $[Pt(NH_3)_2(O^6\text{–}N7\text{-dG})(OH)_2]^+$.

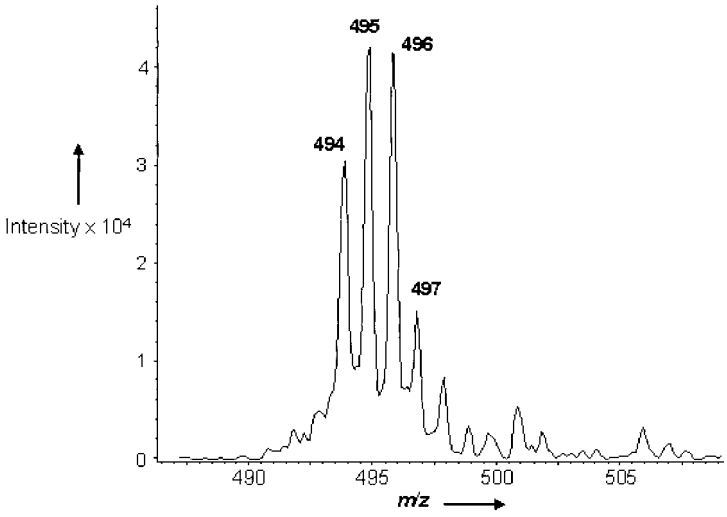


Figure 7. *Offline mass spectrum of cisplatin incubated with dG (molar ratio 1:1, 1 mM) for 6 h at pH 7.4 and 37 °C (Exp. B; see the Experimental Section).*

Table 2. *Calculated m/z ratios of cisplatin–dG adducts.*

Adduct type	Formula	*m/z*
$[Pt(NH_3)_2(dG)]^+$ [a]	$[C_{10}H_{18}N_7O_4Pt]^+$	495
$[Pt(NH_3)_2(dG)OH]^+$	$[C_{10}H_{20}N_7O_5Pt]^+$	513
$[Pt(NH_3)_2Cl(dG)]^+$	$[C_{10}H_{19}ClN_7O_4Pt]^+$	531
$[Pt(NH_3)_2(dG)_2]^+$	$[C_{20}H_{31}N_{12}O_8Pt]^+$	762

[a] O^6–*N*7 chelate.

Table 3. *Calculated m/z values of the oxidized cisplatin–dG adducts.*

Oxidized adduct type	Formula	*m/z*
$[Pt(NH_3)_2(dG)(OH)_2]^+$ [a]	$[C_{10}H_{20}N_7O_6Pt]^+$	529
$[Pt(NH_3)_2(dG)(OH)_3]^+$	$[C_{10}H_{22}N_7O_7Pt]^+$	547
$[Pt(NH_3)_2Cl(dG)(OH)_2]^+$	$[C_{10}H_{21}ClN_7O_6Pt]^+$	565
$[Pt(NH_3)_2(dG)_2(OH)_2]^+$	$[C_{20}H_{33}N_{12}O_{10}Pt]^+$	796

[a] O^6–*N*7 chelate.

Conclusion

A coupled capillary electrophoresis/electrospray-ionization mass spectrometry approach was applied to contribute to the controversial discussion about the existence of an O^6–$N7$ chelate formation between cisplatin and dGMP.

CE/ESI-MS analysis of a 1:1 (molar ratio) mixture of cisplatin and dGMP showed, apart from the bis(dGMP) adduct, a series of monoadducts which could be identified as $[Pt(NH_3)_2Cl(dGMP)]^+$ and $[Pt(NH_3)_2(dGMP)OH]^+$. The structure of a compound with an *m/z* value of 575, which migrated faster than the other adducts, is in accordance with the composition $[Pt(NH_3)_2(dGMP)]^+$. The exchange of dGMP for the closely related analogue dIMP resulted in the formation of corresponding adducts. Due to differences in pK_a values at the *N*1 position of dGMP and dIMP, a significant deviation in relative product distribution was observed, including a peak with an *m/z* value of 560 that was assignable to the corresponding O^6–$N7$ chelate $[Pt(NH_3)_2(O^6$–$N7$-dIMP)$]^+$.

In order to prove the proposed structure and to exclude the formation of other compounds with equivalent *m/z* ratios, for example, an $N7$–αPO_4 macrochelate, dGMP was exchanged for dG in offline ESI-MS experiments. Upon interaction with cisplatin a peak with an *m/z* value of 495, assignable to $[Pt(NH_3)_2(dG)]^+$, was found. Due to the missing phosphate moiety of 2′-deoxyguanosine, the $N7$–αPO_4 macrochelate can be excluded.

Since O^6–$N7$ chelation is described in platinum(IV) chemistry, hydrogen peroxide was added to a cisplatin–dG incubation mixture to trap the O^6–$N7$ platinum(II) chelate and to stabilize it as the corresponding platinum(IV) species. Indeed, an *m/z* value of 529, in accordance with $[Pt(NH_3)_2(O^6$–$N7$-dG)$(OH)_2]^+$, could be detected.

In conclusion, the obtained results indicate that O^6–$N7$ chelation is a possible coordination mode for the interaction of platinum(II) complexes with DNA oxopurine nucleotides and derivatives.

Experimental Section

Due to the controversy about the existence of an O^6–$N7$ chelate and in order to avoid systematic errors, the experiments were performed in two independent laboratories by using two different experimental setups (Exp. A and Exp. B).

Experimental setup A (Exp. A)

Chemicals and sample preparation: 2′-Deoxymononucleotides and cisplatin were purchased from Sigma (Deisenhofen, Germany). Methanol, acetic acid, sodium hydroxide, and ammonia were from Merck (Darmstadt, Germany). All solutions were prepared with purified water taken from a Millipore Milli-Q plus system (Eschborn, Germany) and all chemicals used were of analytical grade. All buffers were degassed by sonication and filtered through 0.45 μm membrane filters (Sartorius, Göttingen, Germany) prior to use. Deoxymononucleotides (dGMP, dIMP) were incubated with cisplatin at 37 °C in a molar ratio of 1:1 to give final concentrations of 0.5 mM.

CE/ESI-MS investigations: CE/ESI-MS analyses were performed by using a Hewlett–Packard ^{3D}Capillary Electrophoresis system equipped with a UV/Vis diode-array detector (at 254 nm) coupled to a Finnigan LCQ mass spectrometer. The background electrolyte (BGE) consisted of 32 mM acetic acid adjusted to pH 9.7 with 25% ammonia solution. Samples were injected into a fused silica capillary that was 76 cm long (22 cm effective length [UV/Vis], 75 μm i.d., 365 μm o.d., kept at 23 °C) by applying 50 mbar pressure for 7 s. Capillaries were preconditioned by standard rinsing procedures (0.1 M sodium hydroxide and BGE for 15 min each prior to use; BGE, sodium hydroxide, and BGE again between separations). At the capillary inlet, voltages of 15–20 kV were applied. After separation within the capillary, the 2′-deoxymononucleotides and platinum adducts were analyzed by utilizing the sheath liquid approach in the positive ionization mode. Sheath liquid consisting of methanol (50%), water (49%), and acetic acid (1%) was delivered at a flow rate of 5–8 μL min^{-1}. The electrospray needle was set at +3.5 kV with respect to the heated sampling capillary, thereby resulting in overall voltages of 11.5–16.5 kV.

Experimental setup B (Exp. B)

Chemicals and sample preparation: Cisplatin was prepared in-house by following the synthetic procedure described elsewhere.[44] 2′-Deoxyguanosine·monohydrate was purchased from Aldrich (Austria). Sodium 2′-deoxyguanosine 5′-monophosphate and ammonium acetate were purchased from Fluka (Buchs, Switzerland). Buffer solutions were prepared with HPLC gradient grade water purchased from Carl Roth (Karlsruhe, Germany). Prior to use all buffer solutions were filtered through 0.2 μm membrane filters (Sartorius, Göttingen, Germany) and degassed in an ultrasonic bath. Cisplatin, 2′-deoxyguanosine, and 2′-deoxyguanosine 5′-monophosphate solutions were prepared in 20 mM ammonium acetate buffer at pH 7.4. Immediately after dissolving and mixing, the samples were incubated at 37 °C.

CE/ESI-MS investigations: CE/ESI-MS measurements were performed on a Hewlett–Packard ^{3D}Capillary Electrophoresis system equipped with a diode-array detector coupled to an esquire$_{3000}$ ion-trap MS^n (Bruker Daltonics, Bremen, Germany) through a sheath-flow electrospray-ionization interface (Bruker Daltonics, Bremen, Germany). Fused-silica capillaries (50 μm i.d., 80 cm total length) were purchased from Polymicro Technologies (Phoenix, AZ, USA). Before the first use, new capillaries were conditioned with 0.1 M sodium hydroxide solution (60 min, HPLC grade; purchased from Fluka, Buchs, Switzerland), water (10 min), and BGE (60 min, 20 mM ammonium acetate buffer at pH 7.4). Prior to each analysis, the capillary was flushed with water (2 min), 0.1 M sodium hydroxide solution (3 min), and BGE (5 min). Capillary and sample-tray temperatures were kept constant at 37 °C. Samples were dissolved in BGE and injected into the capillary at 20 mbar for 20 s. At the capillary inlet, a voltage of 20 kV was applied and kept constant during the analysis. The sheath liquid consisted of methanol (80%), water (19%), and acetic acid (1%) and was delivered at a flow rate of 4 μL min^{-1} by using a 74900 syringe pump from Cole–Parmer Instrument Company (Vernon Hills, IL, USA). Full scan data were acquired by scanning from *m/z* values of 200–1200. The MS parameters optimized for the O^6–$N7$ chelate detection in the CE/ESI-MS experiment (analysis time segment: 0–9 min) are given in Table 4.

Off-line mass spectrometry: The mass spectra were obtained on a Bruker esquire$_{3000}$ ion-trap instrument equipped with an orthogonal ESI source. Samples were directly injected into the mass spectrometer after the appropriate incubation time at 37 °C. Since cisplatin was incubated either with dG or with dGMP, two different sets of acquisition parameters were used (Table 5).

Table 4. *Acquisition parameters for the CE/ESI-MS experiments optimized for the chelate-detection time segment.*

Parameters	Segment 1
time [min]	0–9
polarity	positive
dry gas flow [L min^{-1}]	10
dry gas temperature [°C]	250
nebulizer gas [psi]	10
sheath liquid [μL min^{-1}]	4
HV capillary [V]	3074
skimmer 1 [V]	31.9
capillary exit offset [V]	72
octopole [V]	2.68
octopole Δ [V]	2.4
trap drive	41.1

Table 5. *Offline ESI-MS operating parameters optimized for the analysis of the O^6–N7 chelate formation between cisplatin and dG or dGMP.*

Parameters	Cisplatin + dG	Cisplatin + dGMP
polarity	positive	positive
injection rate [μL min^{-1}]	4	4
HV capillary [V]	3320	3762
dry gas flow [L min^{-1}]	9	9
dry gas temperature [°C]	200	200
nebulizer gas [psi]	10	10
skimmer 1 [V]	31.3	29.4
capillary exit offset [V]	71.8	71.1
octopole [V]	2.89	2.62
octopole Δ [V]	2.4	2.4
trap drive	51.9	35.9

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