Insights into the Reaction of Transplatin with DNA and Proteins: Methionine-Mediated Formation of Histidine–Guanine *trans*-$Pt(NH_3)_2$ Cross-Links

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Abstract: Simultaneous exposure of transplatin to polypeptides and DNA was mimicked by using a model peptide–oligonucleotide conjugate. Initially formed methionine–guanine chelates evolved into adducts with histidine–guanine *trans*-$Pt(NH_3)_2$ cross-links that differed in constitution and stability from those formed by reaction of the same conjugate with the anticancer drug cisplatin. This finding may be due to different capacities of the two diamminedichloroplatinum(II) complexes to interfere with biological processes and may explain their differing cytotoxicities.

Keywords: cross-links · isomers · mass spectrometry · oligonucleotides · peptides · platinum · transplatin

Introduction

Cisplatin, *cis*-diamminedichloroplatinum(II), is an effective antineoplastic drug widely used for the treatment of several cancers.[1] In contrast, the *trans* isomer, transplatin, is without effect. Although it was previously believed that the *cis* configuration was required to generate active complexes, some mononuclear platinum(II) complexes with a *trans* configuration have exhibited cytotoxic activity in tumor cell lines.[2]

The observed inactivity of transplatin remains to be adequately explained. The half-life of monofunctional transplatin–DNA adducts is higher than that of those formed by cisplatin.[3] This may allow monofunctional platinum complexes to be removed from DNA by reaction with glutathione or other sulfur-containing platinum scavengers, or nucleic acid–protein cross-links to be formed.[4] In addition, the distortions induced in DNA by cis- or transplatin complexes are known to be different,[5] both when intra- and interstrand cross-links are formed.[6] Finally, the insolubility of transplatin in aqueous media may reduce its bioavailability.[2c]

In addition to analyses of the structure and properties of DNA–transplatin complexes, studies of the reaction of transplatin with proteins have also been performed. Recent experiments have shown that ubiquitin binds transplatin to form a stable monofunctional complex.[7] The nature of the amino acid involved in the formation of this complex remains to be elucidated, but, in contrast to the reaction with cisplatin, it appears not to be the methionine residue.[7]

Both cis- and transplatin can form DNA–protein cross-links,[8] but their contribution to the toxicity or side effects of the clinically active complex is still under discussion. It has been shown that such cross-links are formed more rapidly by the *cis* than by the *trans* isomer,[8d] and this effect has been related to the contrasting activity of the two complexes. To our knowledge, the nature of the platinum–amino acid linkage formed has yet to be established. More recently, Brabec and co-workers[8e] have described how the DNA–protein cross-links formed by a *trans*-$[PtCl_2\{(E)$-iminoether$\}_2]$ complex may potentiate cell toxicity by interfering with DNA polymerization and repair processes.

Here, we have extended our previous work on the study of the reaction of cisplatin with peptide–oligonucleotide conjugates[9] to transplatin. We studied the interactions established at the molecular level, and compared the behavior of cis- and transplatin from a different perspective.

Results and Discussion

The conjugate used in this study, PhacHisGlyMet-linker–$^{5'}$dCATGGCT (**1**) (Phac = phenylacetyl, Figure 1), was synthesized using solid-phase procedures.[10] Its structure allows the coordinating metal to link different positions of the oligonucleotide moiety to either of the two residues most prone to forming complexes with platinum(II), methionine

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 DOI: 10.1002/chem.200400470

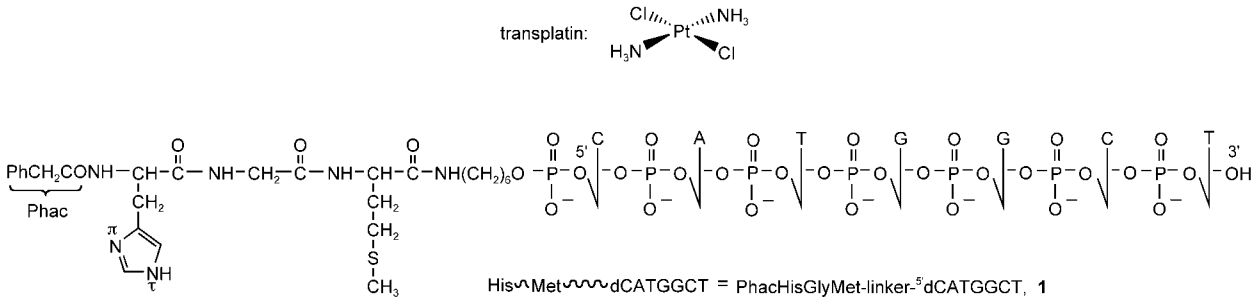


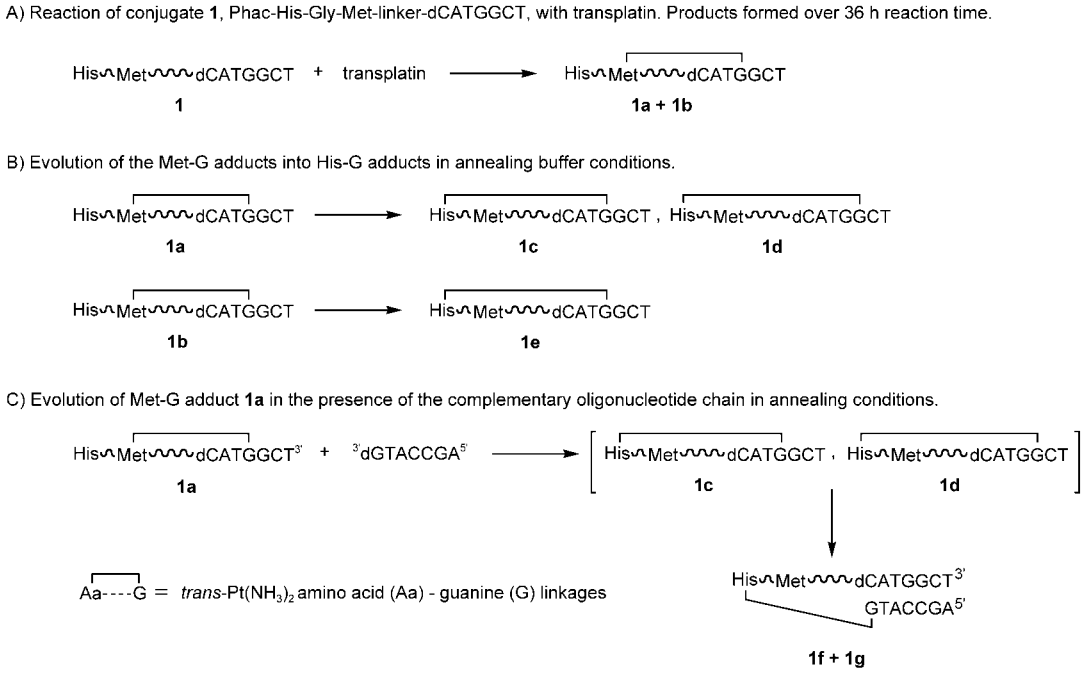
Figure 1. Structures of transplatin and the peptide–oligonucleotide conjugate used in this study.

and histidine. We also studied whether these intramolecular chelates can evolve into intermolecular adducts upon hybridization with the complementary oligonucleotide chain.

A mixture of two products, **1a** and **1b** (ratio ~7:3), was formed relatively rapidly when conjugate **1** reacted with transplatin. These products are constitutionally identical chelates with *trans*-$Pt(NH_3)_2$ linkages between the methionine side chain and the 5′ guanine of the oligonucleotide moiety (Scheme 1A), as inferred from enzymatic digestion with snake venom phosphodiesterase, nuclease S1, papain, and pronase. Coordination of the metal to the thioether, which affords two diastereomers, was also confirmed by the finding that the chelates were not oxidized by H_2O_2. Compounds **1a** and **1b** were quite unstable, as seen by the loss of the $Pt(NH_3)_2$ group and the transformation of both chelates into different products. (Some starting product **1** was always found to accompany the complex isolated after analytical or semipreparative HPLC.)

When stirred for seven days at room temperature in buffer conditions suitable for duplex formation (see below), **1a** afforded two main products, **1c** and **1d**, whereas **1b** gave mainly **1e**. As shown in Scheme 1B, **1c** and **1e** were chelates in which *trans*-$Pt(NH_3)_2$ groups linked the 5′ guanine and the histidine side chain, while in **1d** the metal was coordinated to the 3′ guanine. Chelates **1c** and **1e** most probably differ in the coordination of the metal to either the π or the τ nitrogen atoms of the imidazole ring. As in many experiments with other platinum(II) complexes,[9b,11] the kinetic S–Pt–N adducts have evolved into thermodynamically more stable N–Pt–N adducts.

Adduct **1a**, which was isolated in higher yields than **1b**, was dissolved in the same annealing buffer as above and incubated with the complementary oligonucleotide chain $^{5'}$dAGCCATG at 15 °C. This temperature ensured duplex formation, since the melting temperature of the PhacHis-GlyMet-linker–$^{5'}$dCATGGCT/$^{3'}$dGTACCGA duplex had been identified as 34.1 °C (2 μM duplex concentration).[12] The HPLC trace of the corresponding crude mixture after seven days showed the presence of the thermodynamically stable adducts **1c** and **1d**, the complementary oligonucleotide $^{5'}$dAGCCATG, some conjugate **1** (formed by deplatination of **1a**), and two new products **1f** and **1g**, with platinum



Scheme 1. Evolution of the reactions involving conjugate **1** and its platinated derivatives.

cross-links between the conjugate and the complementary oligonucleotide (Scheme 1C). PAGE analysis showed that the migration of **1f** and **1g** was as expected for molecules containing both oligonucleotide chains (see Figure 2).

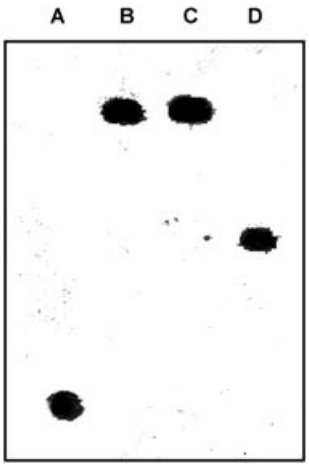
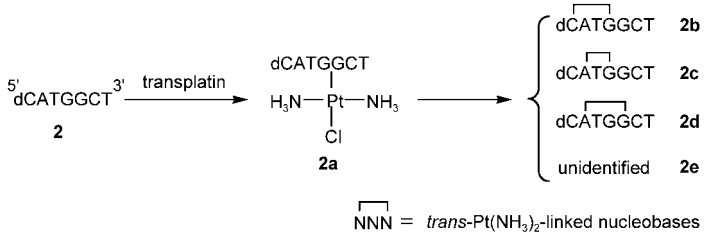


Figure 2. Polyacrylamide gel electrophoresis of *trans*-$Pt(NH_3)_2$ interchain adducts **1f** and **1g** (lanes B and C, respectively). Controls: complementary oligonucleotide $^{3'}$dGTACCGA (lane A) and chelate **1c** (lane D).

In both **1f** and **1g**, the *trans*-$Pt(NH_3)_2$ group linked the N-terminal histidine to the 3′ guanine of the complementary oligonucleotide, indicating that the two original Pt–S and Pt–N(G) linkages of **1a** had been broken to give two new Pt–N linkages. Since we had previously observed migration of platinum from methionine to histidine (Scheme 1B), and detected peaks with retention times corresponding to such adducts (**1c**, **1d**) by HPLC, we surmise that platinum migration from methionine to histidine was the first process to take place. The driving force for the second migration was most probably the formation of a hairpin duplex, which is more stable than those formed by two separate chains.

For comparison purposes, three additional experiments were carried out. On the one hand, the reaction between transplatin and both the oligonucleotide moiety of the conjugate, $^{5'}$dCATGGCT (**2**), and the sulfoxide derivative of conjugate **1** were analyzed. On the other, we studied the reaction between the oligonucleotide (**2**), the peptide (**3**), and transplatin.

In the first of these experiments, **2** was transformed into a monofunctional adduct, **2a**, which slowly evolved into a mixture of four products, **2b**–**2e** (Scheme 2). Three of these



Scheme 2. Evolution of the reaction of transplatin with oligonucleotide **2**.

products were chelates in which *trans*-$Pt(NH_3)_2$ units linked one of the guanines and either one cytosine (**2b**) or one adenine (**2c**, **2d**). The structure of the fourth chelate (**2e**) could not be unambiguously inferred from the data. The reaction of the sulfoxide derivative of conjugate **1** with transplatin gave adducts with the same structure as those derived from **2** (data not shown). No histidine–Pt–guanine linkages were formed.

The evolution of these reactions differs markedly from that of conjugate **1**, since neither monofunctional adducts nor *trans*-$Pt(NH_3)_2$-linked nucleobases were found in the case of the conjugate. We conclude that transplatin first reacts with the thioether group of methionine, which renders the *trans* ligand so labile and reactive that we can only detect the resulting chelates, and that these evolve into histidine–guanine adducts rather than adducts with two metal-linked nucleobases.

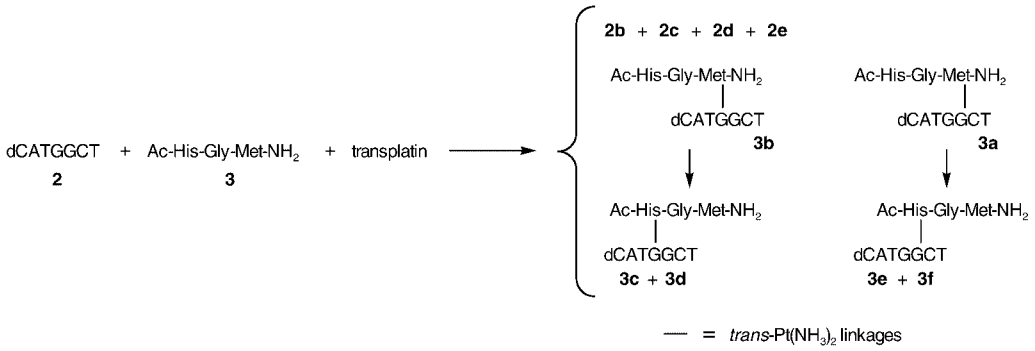
This suggests that the progress of the reaction of DNA with transplatin may depend on whether sulfur-containing functional groups are close to the DNA reaction site. If the metal complex reaches and reacts with guanines (or, eventually, other nucleobases), the resulting monofunctional adducts can either evolve into intra- or interstrand cross-links or be cleaved by reaction with sulfur-containing amino acids (or other sulfur-containing metal scavengers). Alternatively, bifunctional adducts formed by reaction of the metal with a methionine side chain and guanine may be subsequently transformed into new complexes with more stable bonds, cross-linking either the two DNA chains or one DNA strand and a protein.

The reaction between the three separate components to which we have referred above (see Scheme 3) afforded a mixture of different products. Besides the adducts **2b**–**2e**, it was shown to contain products with *trans*-$Pt(NH_3)_2$ units linking methionine and one of the two guanines, **3a** and **3b**, which evolved into histidine–guanine cross-linked adducts (**3c**–**3f**). The evolution of this reaction was the same as that observed for conjugate **1**, showing that the methionine-mediated formation of histidine–guanine cross-links can also take place in an intermolecular process.

Finally, it is interesting to note that cis- and transplatin generate different adducts when exposed simultaneously to peptide and oligonucleotide chains, as can be seen by comparing the structures of adducts **1c**–**1e** (Scheme 1) with those formed when **1** was treated with cisplatin (Figure 3).[9b] The two types of adduct differ not only in structure but also in stability, since the trifunctional adducts formed by cisplatin are more stable than complexes **1c**–**1e**. No interchain linkage was formed when one of the tricoordinated adducts (**4b**) and the complementary oligonucleotide chain were mixed under annealing conditions (data not shown).

Conclusion

In summary, transplatin may react with DNA to yield monofunctional adducts that may either be repaired or form intra- or interchain adducts; however, as shown here, it may also cross-link DNA to proteins, preferably through guanine



Scheme 3. Products formed upon reaction of the peptide and the oligonucleotide with transplatin.

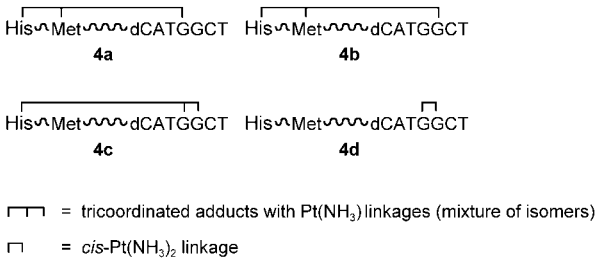


Figure 3. Adducts formed by reaction of conjugate **1** with cisplatin.

and histidine moieties. Besides other known factors stated earlier, the different structure and stability of the DNA–protein adducts formed may also account for the different biological activities of cis- and transplatin. The fact that cisplatin generates more stable DNA–protein cross-links may contribute to the hijacking of proteins away from their target site, as well as to the interference with cell repair mechanisms. In this respect, we would like to refer to the recent suggestion[8e] that variable efficiency in forming DNA–protein cross-links may explain the variable activity of different platinum(II) complexes.

Experimental Section

General procedures: Transplatin was solubilized by heating to 80–90 °C in a water bath with continuous vortexing until complete dissolution was achieved. The required amount of the resulting solution was immediately added to the solution of the hybrid to avoid precipitation of the platinum compound. All reactions were carried out in 0.5–1.5 mL Eppendorf tubes as described below.

The evolution of reactions was monitored by reversed-phase HPLC with on-line UV detection. Peaks with the same retention time between the different runs were collected, frozen immediately, and lyophilized. HPLC was carried out on Kromasil C18 columns (250 × 4 mm, 10 μM, 1 mL min^{-1}), eluting with aqueous ammonium acetate (0.01 M) as solvent A and a 1:1 mixture of acetonitrile and water as solvent B (linear gradient from 10 to 40 % of B in 30 min, and from 40 to 100 % of B in 5 min). Samples from reactions in duplex-forming conditions were desalted by elution through Sep–Pak cartridges (Phase Separations Ltd.) prior to HPLC analysis.

PAGE electrophoresis was performed under denaturing conditions (7 M urea) on a 20 % polyacrylamide gel, at 500–750 V for 3–4 h. Detection was carried out by reaction with Stains-All dye (Sigma).

Structural information was obtained from treatment with different enzymes (phosphodiesterases or proteases), or by reaction with H_2O_2, followed by mass spectrometric analysis (negative mode). Matrix-assisted laser desorption ionization-time-of-flight (MALDI-TOF) mass spectrometric analysis was carried out using a Voyager-DE RP instrument (Perceptive Biosystems). Ionization of platinum adducts was carried out in the negative mode using 2′,4′,6′-trihydroxyacetophenone (10 mg in 1 mL ACN/H_2O, 1:1 v/v) as a matrix in the presence of ammonium citrate (50 mg in 1 mL H_2O). In general, 0.02–0.1 OD units of the platinum adduct were solubilized in 20 μL water for characterization assays. Subsequently, 1 μL ammonium citrate solution was added to 1 μL sample solution, and then 1 μL matrix solution. After mixing by withdrawing and expelling the solution with a pipette several times, 1 μL of the resulting solution was spotted onto the sample plate and dried. Typical isotopic distribution for platinum-containing compounds was clearly observed with the use of a reflector (analysis in the linear mode had poorer resolution and gave a broad peak). In all experiments, the highest isotopic peak was taken as $[M-H]^-$. When electrospray mass spectrometry was used (VG Quattro, Micromass), samples were dissolved in a 1:1 v/v acetonitrile/water mixture containing 1 % triethylamine. Calculated mass values for neutral compounds (M) are indicated in all cases.

Digestion with snake venom phosphodiesterase (SVPD) (Boehringer-Mannheim, 1.5 U/500 μL; EC 3.1.4.1): A solution of the platinum adduct (3 μL) was incubated with Tris-HCl buffer pH 8.0 (0.5 μL, 0.1 M), $MgCl_2$ (0.2 μL, 0.1 M), and SVPD (0.5 μL) for 30 min at 37 °C.

Digestion with calf spleen phosphodiesterase (Sigma, 10.3 U mg^{-1}; EC 3.1.16.1): A solution of the platinum adduct (3 μL) was incubated with 0.5 μL of a solution of the enzyme (0.34 mg in 50 μL 0.2 M ammonium acetate buffer, pH 5.4), for 3 h at 37 °C.

Digestion with nuclease S1 (from *Aspergillus oryzae*, Pharmacia Biotech, 343 U μL^{-1}): A solution of the platinum adduct (3 μL) was incubated with 0.5 μL of nuclease S1 buffer (50 mM NaCl, 50 mM ammonium acetate, 5 mM $ZnCl_2$, pH 4.6) and nuclease S1 solution (0.5 μL: 0.5 μL in 373 μL H_2O), for 15 min at 37 °C.

Digestion with papain (from papaya latex, 14 U mg^{-1}, EC 3.4.22.2): A solution of the platinum adduct (3 μL) was incubated with 0.5 μL of a solution of papain (2 mg in 10 μL water) and ammonium acetate buffer (0.5 μL, 0.1 M pH 5.4) for 3 h at 37 °C.

In some cases, nucleoside composition was determined by digestion with nuclease S1 and alkaline phosphatase (Sigma, 0.23 U μL^{-1}; EC 3.1.3.1). 0.02 OD units were dissolved in water (2 μL), followed by the addition of nuclease S1 buffer (2 μL), a solution of nuclease S1 (2 μL), and alkaline phosphatase (2 μL). The sample was incubated at 37 °C for 20 h. The identity and ratio of nucleosides were determined by HPLC analysis using the following linear gradient: isocratic 10 % of B for 5 min and from 10 to 25 % of B for 15 min. Retention times (t_R) of the nucleosides: dC 2.9, dG 4.9, T 7.0, and dA 10.3 min.

Reaction between conjugate 1 and transplatin: Peptide–oligonucleotide conjugate PhacHisGlyMet-linker–$^{5'}$dCATGGCT (**1**) (5 OD units) was dissolved in water (488 μL) at 37 °C. A 10 mM solution of transplatin (11.7 μL, 1.5 equiv) was added and the reaction mixture was maintained at 37 °C. The concentration of **1** in the resulting solution was 0.15 mM. Different aliquots were separated from the reaction mixture at different times and, after addition of saturated aqueous KCl, kept frozen prior to analysis and purification.

The evolution of the hybrid-transplatin reaction was monitored by reversed-phase HPLC with on-line UV detection. Two major new products, **1a** and **1b** (approximate ratio 7:3), were identified, isolated, and characterized. These eluted at 20.9 and 17.1 min, respectively (retention time of **1**: 18.2 min).

Characterization of adducts 1a and 1b: MALDI-TOF mass spectrometric analysis indicated, in both cases, coordination of *trans*-$Pt(NH_3)_2$ groups to the hybrid molecule: **1a** *m/z*: 2943.43 $[M-H]^-$ and **1b** *m/z*: 2943.54 $[M-H]^-$ (monoisotopic calculated mass for [hybrid+$Pt(NH_3)_2$−2H]: 2944.6).

The absorption maximum of both **1a** and **1b** was shifted from 260 nm (in **1**) to 262 nm.

Treatment with snake venom phosphodiesterase: In both adducts **1a** and **1b**, removal of the fragment pGpCpT indicated coordination to the 5′G nucleobase of the oligonucleotide chain (*m/z*: 2021.33 $[M-H]^-$ in the product formed from **1a**, and *m/z*: 2022.21 $[M-H]^-$ in that obtained from **1b**; monoisotopic calculated mass for [hybrid−pGpCpT+$Pt(NH_3)_2$−2H]: 2022.5).

Nuclease S1 digestion of chelates with a peptide–Pt–oligonucleotide linkage afforded, amongst others, a product with an *m/z* ratio 18 units higher than that of the parent compound. This corresponds to cleavage of a single phosphodiester bond with the subsequent addition of one water molecule. In both hybrid–transplatin adducts, nuclease S1 treatment afforded products with an [*M*+18] mass: *m/z*: 2965.53 $[M-H]^-$ after digestion of **1a**, and *m/z*: 2965.17 $[M-H]^-$ after digestion of **1b**.

No reaction with aqueous H_2O_2 (5% v/v solution, 2 h at room temperature) confirmed coordination of platinum to sulfur in both adducts. After H_2O_2 treatment, **1a** *m/z*: 2944.88 $[M-H]^-$, and **1b** *m/z*: 2944.63 $[M-H]^-$.

Removal of the fragment Phac-His-Gly upon reaction with papain indicated no coordination of platinum to the imidazole ring in either chelate. MS data of the adducts resulting from digestion with papain: *m/z*: 2638.49 $[M-H]^-$ (from **1a**), and *m/z*: 2629.83 $[M-H]^-$ (from **1b**); monoisotopic calculated mass for [hybrid−Phac-His-Gly+$Pt(NH_3)_2$−3H]: 2632.4.

Evolution of chelates 1a and 1b: Chelates of **1a** or **1b** (0.1 OD units) were dissolved in an aqueous neutral buffer (800 μL) suitable for hybridization: NaCl (200 mM), $MgCl_2$ (5 mM), and NaH_2PO_4/Na_2HPO_4 (10 mM), pH 7.0. Samples were kept at 20 °C for seven days, desalted with a Sep–Pak column, and analyzed by HPLC.

Chelate **1a** (t_R 20.9 min) generated two major products: **1c** (t_R 20.9 min) and **1d** (t_R 25.6 min), in an approximate ratio of 7:3. Compound **1c** was confirmed to be different from **1a**, in spite of the fact that both had the same retention time under the HPLC analysis conditions used. Compound **1c** was slowly transformed into **1d**.

Chelate **1b** (t_R 17.1 min) was transformed into new products with higher retention times. The major new adduct **1e** (t_R 22.6 min) could be fully characterized.

Some free hybrid was also detected in both cases due to deplatination.

Characterization of adducts 1c, 1d, and 1e: Adducts **1c**, **1d**, and **1e** were identified as *trans*-$Pt(NH_3)_2$-containing adducts by MALDI-TOF mass spectrometry: *m/z*: 2943.91 $[M-H]^-$ for **1c**, *m/z*: 2942.24 $[M-H]^-$ for **1d**, and *m/z*: 2945.21 $[M-H]^-$ for **1e** (monoisotopic calculated mass for [hybrid+$Pt(NH_3)_2$−2H]: 2944.6).

The absorption maximum of **1c**, **1d**, and **1e** was shifted from 260 nm (in **1**) to 262 nm.

Fragment pGpCpT was eliminated upon treatment of **1c** and **1e** with snake venom phosphodiesterase, which indicated coordination to the 5′G nucleobase. MS data of the digestion products: *m/z*: 2023.19 $[M-H]^-$ (from **1c**), *m/z*: 2024.75 $[M-H]^-$ (from **1e**) (monoisotopic calculated mass for [hybrid−pGpCpT+$Pt(NH_3)_2$−2H]: 2022.5). In the case of adduct **1d**, elimination of fragment pCpT indicated coordination to the 3′G nucleobase, *m/z*: 2353.72 $[M-H]^-$ (monoisotopic calculated mass for [hybrid−pCpT+$Pt(NH_3)_2$−2H]: 2351.5).

Stability to papain treatment indicated coordination of platinum to the imidazole ring in all compounds (Pt–Nim bonds render compounds stable to this enzyme). After papain treatment, **1c** *m/z*: 2945.23 $[M-H]^-$, **1d** *m/z*: 2943.75 $[M-H]^-$, **1e** *m/z*: 2941.84 $[M-H]^-$.

H_2O_2 treatment of **1c**, **1d**, and **1e** gave *m/z* ratios 16 units higher than the parent species, corresponding to oxidation of the "unprotected" thioether to sulfoxide and indicating no coordination to the thioether group. MS data of the oxidized products: *m/z*: 2959.03 $[M-H]^-$ (oxidized **1c**), *m/z*: 2961.72 $[M-H]^-$ (oxidized **1d**), and *m/z*: 2959.32 $[M-H]^-$ (oxidized **1e**) (monoisotopic calculated mass for [hybrid+$Pt(NH_3)_2$−2H+16]: 2960.6).

Hybridization of adduct 1a with the complementary oligonucleotide chain: Chelate **1a** (3 OD units, 0.047 μmol) and the complementary oligonucleotide chain, $^{3'}$dGTACCGA (3.28 OD units, 1 equiv) were dissolved in the same buffer (11.75 mL) as above. Duplex concentration was 4 μM. The solution was heated at 37 °C for 1 h, cooled slowly to 20 °C, and kept in a water bath at 10–15 °C for nine days with continuous stirring. The mixture was desalted through a Sep–Pak column and analyzed by HPLC. Six main peaks were identified, collected, and characterized by MALDI-TOF mass spectrometry: t_R 9.7 min, complementary oligonucleotide $^{3'}$dGTACCGA; t_R 13.5 min, adduct with an interchain cross-link **1f**; t_R 14.2 min, adduct with an interchain cross-link **1g**; t_R 19.2 min, conjugate **1**; t_R 21.2 min, chelate **1c**; and t_R 26.2 min, chelate **1d**. The ratio of adducts **1f** to **1g** in the reaction mixture was 3:7. Both **1f** and **1g** were adducts with *trans*-$Pt(NH_3)_2$ interchain linkages between the histidine residue of **1** and the 3′-terminal guanine of the complementary chain, as shown by mass spectrometric analysis after digestion with snake venom or calf spleen phosphodiesterases, oxidation with H_2O_2, and nucleoside composition (see below).

Characterization of adducts 1f and 1g: Data for **1f**: MALDI-TOF MS *m/z*: 5052.67 $[M-H]^-$ and 2526.67 $[M-2H]^{2-}$; electrospray MS *m/z*: 1261.3 $[M-4H]^{4-}$, 1009.4 $[M-5H]^{5-}$, 720.2 $[M-7H]^{7-}$, calculated mass M: 5050.27 ± 1.67. Data for **1g**: MALDI-TOF MS *m/z*: 5054.77 $[M-H]^-$, 2530.87 $[M-2H]^{2-}$; electrospray MS *m/z*: 1260.3 $[M-4H]^{4-}$, 1009.2 $[M-5H]^{5-}$, 840.3 $[M-6H]^{6-}$, 720.2 $[M-7H]^{7-}$, 630.4 $[M-8H]^{8-}$, calculated mass M: 5049.56 ± 1.55. Calculated mass for [hybrid+$^{3'}$dGTACCGA+$Pt(NH_3)_2$−2H]: 5051.5 (average).

MALDI-TOF mass spectrometric analysis after H_2O_2 treatment of both adducts gave an *m/z* ratio 16 units higher than that of the parent compounds, indicating no coordination to the thioether group. MS data: *m/z*: 5070.03 $[M-H]^-$ and 2535.06 $[M-2H]^{2-}$ (oxidized **1f**); *m/z*: 5072.00 $[M-H]^-$ and 2538.85 $[M-2H]^{2-}$ (oxidized **1g**).

Stability to papain indicated coordination of platinum to the imidazole ring in both adducts. After papain treatment, **1f** *m/z*: 5063.8 $[M-H]^-$, and **1g** *m/z*: 5054.76 $[M-H]^-$.

Fragment pCpApTpGpGpCpT was eliminated upon treatment of both adducts with snake venom phosphodiesterase, which indicated no coordination to the oligonucleotide chain of conjugate **1**. New products formed: *m/z*: 2887.80 $[M-H]^-$ (from **1f**), and *m/z*: 2887.85 $[M-H]^-$ (from **1g**); monoisotopic calculated mass for [hybrid+$^{3'}$dGTACCGA+$Pt(NH_3)_2$−$^{5'}$pCpApTpGpGpCpT−2H]: 2891.7. Elimination of fragment $^{5'}$ApGpCpCpApTp upon calf spleen phosphodiesterase treatment indicated coordination to the 3′G nucleobase of the complementary oligonucleotide chain. New products formed: *m/z*: 3211.42 $[M-H]^-$ (from **1f**), and *m/z*: 3209.07 $[M-H]^-$ (from **1g**); monoisotopic calculated mass for [hybrid+$^{3'}$dGTACCGA+$Pt(NH_3)_2$−$^{5'}$ApGpCpCpApTp−2H]: 3211.7.

Nucleoside composition: **1f**: dC/dG/T/dA 4.25:4.08:3.24:3.16, and **1g**: dC/dG/T/dA 4.37:3.82:3.07:2.73 (expected ratio for the duplex: 4:4:3:3).

Reaction of oligonucleotide $^{5'}$dCATGGCT (2) with transplatin: The oligonucleotide $^{5'}$dCATGGCT (**2**) (3 OD units) was dissolved in water (580 μL) at 37 °C. A 10 mM aqueous solution of transplatin (5.2 μL, 1.1 equiv) was added, and the reaction was maintained at 37 °C. Different aliquots were separated from the reaction mixture at different times and, after addition of saturated aqueous KCl solution, kept frozen prior to analysis and purification by reversed-phase HPLC.

HPLC traces after 2 h reaction time showed two main products; the starting oligonucleotide **2** (t_R 16.1 min, 53%) and a new product **2a** (t_R 17.7 min, 25%). Four new compounds were subsequently formed from **2** and **2a**, eluting at 13.1 min, **2b**; 14.0 min, **2c**; 14.4 min, **2d**; and 15.0 min, **2e**. Compound **2a** was no longer detected after five days, and the ratios of **2b**–**2e** were shown to be constant (36%, 15%, 19%, and 30%, respectively).

MALDI-TOF mass spectrometric analysis of the five new compounds indicated transplatin coordination to the oligonucleotide moiety. Com-

pound **2a** was shown to be a monofunctional adduct incorporating a $Pt(NH_3)_2Cl$ group, while all the other compounds contained $Pt(NH_3)_2$ moieties.

Characterization of adducts 2a, 2b, 2c, 2d, and 2e: MALDI-TOF MS: **2a**: *m/z*: 2359.20 $[M-H]^-$ (monoisotopic calculated mass for [oligonucleotide+$Pt(NH_3)_2Cl-1H$]: 2358.4); **2b** *m/z*: 2322.13 $[M-H]^-$; **2c** *m/z*: 2322.18 $[M-H]^-$; **2d** *m/z*: 2321.87 $[M-H]^-$; **2e** *m/z*: 2322.13 $[M-H]^-$ (monoisotopic calculated mass for [oligonucleotide+$Pt(NH_3)_2-2H$]: 2322.4).

In chelates **2b** and **2c**, treatment with snake venom phosphodiesterase removed the fragment pGpCpT, which indicated coordination to the 5′G nucleobase (*m/z*: 1402.04 $[M-H]^-$ in the digestion product from **2b**, and *m/z*: 1402.71 $[M-H]^-$ in that obtained from **2c**; monoisotopic calculated mass for [oligonucleotide−pGpCpT+$Pt(NH_3)_2-2H$]: 1400.3). In the case of adducts **2d** and **2e**, elimination of fragment pCpT indicated coordination to the 3′G nucleobase (*m/z*: 1732.61 $[M-H]^-$ in the product obtained from **2d**, and *m/z*: 1731.23 $[M-H]^-$ in that obtained from **2e**; monoisotopic calculated mass for [oligonucleotide−pCpT+$Pt(NH_3)_2-2H$]: 1729.3).

Stability of adduct **2b** to digestion with calf spleen phosphodiesterase indicated coordination of platinum to the 5′C nucleobase: *m/z*: 2321.00 $[M-H]^-$ (monoisotopic calculated mass for [oligonucleotide+$Pt(NH_3)_2-2H$]: 2322.4). In chelates **2c** and **2d**, removal of Cp indicated coordination to the 5′A nucleobase (*m/z*: 2032.52 $[M-H]^-$ in the product obtained from **2c**, and *m/z*: 2032.79 $[M-H]^-$ in that resulting from digestion of **2d**; monoisotopic calculated mass for [oligonucleotide−Cp+$Pt(NH_3)_2-2H$]: 2033.4). In the case of adduct **2e**, the *m/z* ratios detected (*m/z* 1419.77, 1748.77, and 2035.60) did not allow the coordination pattern to be unambiguously established. The *m/z* value of 1419.77, which corresponds to removal of the fragment CpApTp and indicates coordination to the 5′G, and the *m/z* value of 2035.60, which corresponds to removal of Cp and indicates coordination to adenine, suggest that a chelate with a *trans*-$Pt(NH_3)_2$ between adenine and the 5′G, as in the case of **2c**, might have been formed. We have no explanation for the 1748.77 *m/z* value, which does not fit with the previous data.

Reaction of oligonucleotide ^{5}dCATGGCT (2) and tripeptide Ac-His-Gly-Met-NH_2 (3) with transplatin: The oligonucleotide ^{5}dCATGGCT (**2**) (3 OD units) and tripeptide Ac-His-Gly-Met-NH_2 (**3**) (1 equiv) were dissolved in water (535 μL) at 37 °C. A 10 mM aqueous solution of transplatin (4.5 μL, 0.95 equiv) was added, and the reaction was maintained at 37 °C. Different aliquots were separated from the reaction mixture at different times and, after addition of saturated aqueous KCl solution, kept frozen prior to analysis and purification by reversed-phase HPLC.

HPLC traces after 24 h reaction time showed two sets of products besides the starting oligonucleotide **2** (t_R 15.6 min) and tripeptide **3** (t_R 17.2 min): the same oligonucleotide–transplatin adducts **2b**–**2e** arising from reaction between **2** and transplatin, and six new compounds eluting at 16.9 min, **3a**; 17.7 min, **3b**; 18.8 min, **3c**; 19.6 min, **3d**; 21.0 min, **3e**; and 21.3 min, **3f**. With longer reaction times, compounds **3a** and **3b** were completely transformed into **3c**–**3f**, whose relative ratio was kept constant (31%, 13%, 19%, and 37%, respectively). Subsequent experiments demonstrated that **3b** was transformed into **3c** and **3d**, and that **3a** evolved into **3e** and **3f**.

MALDI-TOF mass spectrometric analysis of the six new compounds indicated transplatin coordination to both the oligonucleotide and the peptide moieties.

Characterization of adducts 3a, 3b, 3c, 3d, 3e, and 3f: MALDI-TOF MS: **3a** *m/z*: 2705.68 $[M-H]^-$; **3b** *m/z*: 2705.49 $[M-H]^-$; **3c** *m/z*: 2706.20 $[M-H]^-$; **3d** *m/z*: 2706.06 $[M-H]^-$; **3e** *m/z*: 2705.82 $[M-H]^-$; **3f** *m/z*: 2705.73 $[M-H]^-$; (monoisotopic calculated mass for [peptide+oligonucleotide+$Pt(NH_3)_2-2H$]: 2706.6).

The absorption maximum of **3a**–**3f** was shifted from 260 nm (in **2**) to 261 nm.

No reaction with aqueous H_2O_2 (5% v/v solution, 2 h at room temperature) confirmed coordination of platinum to sulfur in **3a** and **3b**. H_2O_2 treatment of **3c**, **3d**, **3e,** and **3f** gave *m/z* ratios 16 units higher than that of the parent species, indicating no coordination to the thioether group. MS data of the new products: *m/z*: 2720.22 $[M-H]^-$ (oxidized **3c**), *m/z*: 2720.82 $[M-H]^-$ (oxidized **3d**), *m/z*: 2720.78 $[M-H]^-$ (oxidized **3e**), and *m/z*: 2720.49 $[M-H]^-$ (oxidized **3f**) (monoisotopic calculated mass for [peptide+oligonucleotide+$Pt(NH_3)_2-2H+16$]: 2722.6).

In chelates **3c** and **3d**, treatment with snake venom phosphodiesterase removed the fragment pGpCpT, which indicated coordination to the 5′G nucleobase (*m/z*: 1783.66 $[M-H]^-$ in the digestion product from **3c**, and *m/z*: 1783.74 $[M-H]^-$ in that obtained from **3d**; monoisotopic calculated mass for [peptide+oligonucleotide−pGpCpT+$Pt(NH_3)_2-2H$]: 1784.4). In the case of adducts **3e** and **3f**, fragment pCpT was eliminated, indicating coordination to the 3′G nucleobase (*m/z*: 2112.40 $[M-H]^-$ in the product obtained from **3e**, and *m/z*: 2113.57 $[M-H]^-$ in that obtained from **3f**; monoisotopic calculated mass for [peptide+oligonucleotide−pCpT+$Pt(NH_3)_2-2H$]: 2113.5).

In chelates **3c** and **3d**, removal of CpApTp by calf spleen phosphodiesterase confirmed coordination to the 5′G nucleobase (*m/z*: 1802.68 $[M-H]^-$ in the case of digestion of **3c**, and *m/z*: 1801.21 $[M-H]^-$ when the treatment was carried out on **3d**; monoisotopic calculated mass for [peptide+oligonucleotide−CpApTp+$Pt(NH_3)_2-2H$]: 1800.4). In the case of adducts **3e** and **3f**, coordination to the 3′G nucleobase was confirmed by removal of the fragment CpApTpGp by the same 5′-exonuclease (*m/z*: 1471.12 $[M-H]^-$ in the reaction with **3e**, and *m/z*: 1476.07 $[M-H]^-$ in the reaction with **3f**; monoisotopic calculated mass for [peptide+oligonucleotide−CpApTpGp+$Pt(NH_3)_2-2H$]: 1471.4).

Stability to papain indicated coordination of platinum to the imidazole ring in **3c**–**3f**. After papain treatment, **3c** *m/z*: 2705.72 $[M-H]^-$, **3d** *m/z*: 2705.82 $[M-H]^-$, **3e** *m/z*: 2705.60 $[M-H]^-$, and **3f** *m/z*: 2705.78 $[M-H]^-$.

Acknowledgments

This work was supported by funds from the Ministerio de Ciencia y Tecnología (BQU2001-3693-C02-01) and the Generalitat de Catalunya (2001SGR-49 and Centre de Referència de Biotecnologia).

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Received: May 13, 2004
Published online: September 23, 2004

