Kinetic Analysis of the Reactions between GG-Containing Oligonucleotides and Platinum Complexes. 1. Reactions of Single-Stranded Oligonucleotides with *cis*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ and $[Pt(NH_3)_3(H_2O)]^{2+}$

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Using concentration measurements based on high performance liquid chromatography, we have investigated the kinetics of reaction between single-stranded oligonucleotides containing a d(GpG) sequence, i.e., d(GG), d(TGG), d(TTGG), and d(CTGGCTCA), and the platinum complexes *cis*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ (**1**) and $[Pt(NH_3)_3(H_2O)]^{2+}$ (**2**). The rate constants for the substitution of one aqua ligand of platinum in **1** or **2** by each guanine of the oligonucleotides were individually measured, as well as, for **1**, those for the subsequent conversion of the monoadducts to the diadduct. For the platination of d(GG) and d(TGG), the rate constants are similar for the 5′- and 3′-guanines. The longer oligonucleotides d(TTGG) and d(CTGGCTCA) are platinated slightly faster on the 5′-G than on the 3′-G. **2** shows a similar slight preference for the 5′-guanine, but it reacts by a factor of 4−10 more slowly than **1**. For both complexes, the platination rate constants increase with increasing oligonucleotide length. Platination of the 5′-G by **1** is 1 order of magnitude faster on d(CTGGCTCA) than on d(GG). Concerning the chelation step giving the GG diadduct of **1**, the longer the oligonucleotide, the larger is the ratio between the rates of the cyclization of the 3′- and 5′-monoadducts $k_{3'c}$ and $k_{5'c}$: $k_{3'c}/k_{5'c}$ equals 1.4 for d(GG) and 3.3 for d(CTGGCTCA).

Introduction

Cisplatin is one of the most effective anticancer drugs currently used worldwide.[1] Biological and chemical experiments have indicated that the interaction of cisplatin with DNA is responsible for its antineoplastic activity.[2−6] The major lesion, the GG intrastrand cross-link, accounts for 60−65% of platinum bound to DNA.[7−11] This is roughly 2 times more than what would be expected if all guanines were equireactive (the probability that a given guanine has another guanine as a neighbor is 37% in salmon sperm DNA, for which the adduct analyses were carried out). Since the binding of cisplatin to DNA is under kinetic control,[12,13] the overstatistical platination of GG sequences suggests that guanines involved in G_n runs ($n \geq 2$) are more reactive than other guanines.

In cisplatin-treated ovarian cancer patients, the extent of formation of the AG and GG intrastrand cross-links in leukocyte DNA correlated with the treatment response.[14] This result suggests that the GG and AG adducts are somewhat related to anticancer activity, although it does not prove that they are at the origin of the activity. In *Escherichia coli*, the GG adducts are indeed cytotoxic.[15] The second major adduct, the AG intrastrand cross-link (∼20% of DNA-bound platinum) [7−11] is more mutagenic in *E. coli* than the GG cross-link.[15−17] On the basis of these observations, Bradley et al. have suggested that it may be desirable to design platinum antitumor drugs with reduced propensity to form AG cross-links.[15] Such design requires an understanding of the sequence selectivity of cisplatin binding. Our objective is to achieve this understanding. The strategy we have been pursuing is to determine the platination rate constants for guanines in different contexts, in single- and double-stranded oligonucleotides, and from the comparison of these rate constants to elucidate the role of the neighboring residues in the oligonucleotide as well as that of the platinum ligands. This should give us finally the clue to the selective binding of cisplatin to GG sequences.

A key point in this study is to determine the platination rate constants for the two guanines of a GG sequence separately, in order to see whether the reactivity of only one of them or that of both is enhanced. This separate determination of the platination rate constants requires the measurement of the monoadducts' concentrations. The platination rate constants can be roughly determined from the slopes of the monoadduct concentration curves at initial time. A more accurate method

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† This work is part of a long-term program and involves major contributions of two Ph.D. students: F.G. and F.R.

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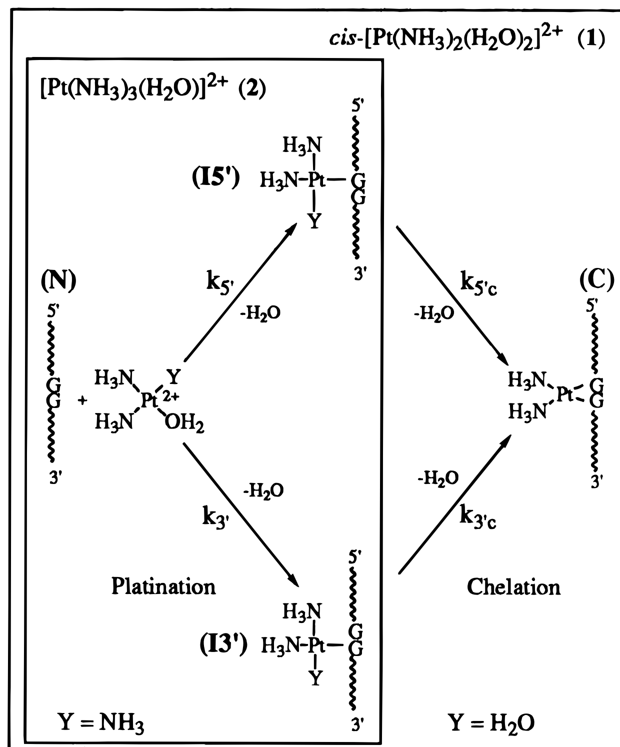

Figure 1. Kinetic scheme for binding of *cis*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ (Y = H_2O) and $[Pt(NH_3)_3(H_2O)]^{2+}$ (Y = NH_3) complexes to a GG sequence of an oligonucleotide.

consists of measuring the concentrations of all the four species participating in the two-step process (Figure 1), *i.e.*, the unplatinated oligonucleotide (**N**), the two monoadducts (intermediates **I5′** and **I3′**), and the final diadduct (chelate **C**), and fitting all concentration curves using an iterative program, optimizing the two platination and the two chelation rate constants. Such a procedure, using a KCl or KBr quenching reagent to arrest the reaction and HPLC as the analytical technique, has been delineated in a previous communication.[18]

In this account, we present the results of a kinetic analysis of the stoichiometric reaction between *cis*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ (**1**), the doubly-aquated cisplatin form, and the oligonucleotides d(GG), d(TGG), d(TTGG), and d(CTGGCTCA). This work enabled us to study the influence of the chain length on the first platination and chelation rate constants. The platination rate constants obtained with $[Pt(NH_3)_3(H_2O)]^{2+}$ (**2**) revealed the influence of the "inert" ammine ligand of platinum compared to the aqua ligand. The present work is prerequisite for the more complex analysis of the reactions of a duplex oligonucleotide, which will be the subject of a future paper.

Experimental Section

Starting Materials and Sample Preparation. d(GG) was obtained from Sigma Chemicals. d(TGG), d(TTGG), and d(CTGGCTCA) were synthesized *via* an improved phosphodiester method as ammonium salts[19] by J. Igolen and co-workers (Institut Pasteur, Paris). Approximate concentrations of oligonucleotides were evaluated photometrically. The molar absorption coefficients of the oligonucleotides were approximated according to Cantor and Warshaw.[20] *cis*-$[PtCl_2(NH_3)_2]$ was kindly provided by Rhône-Poulenc Rorer (Vitry-sur-Seine, France). A solution of *cis*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ was prepared by dissolving a suspension of *cis*-$[Pt(NO_3)_2(NH_3)_2]$[21] in water. A solution of $[Pt(NH_3)_3(H_2O)]^{2+}$ was prepared from $[PtCl(NH_3)_3]NO_3$ by an adapted version of the method by Morita and Bailar.[22,23] Perchloric acid and all salts were purchased from Merck. Exonuclease venum phosphodiesterase (VPD) was purchased from Sigma.

The reactions were initiated by mixing a solution of approximately 2 μmol of d(GG), d(TGG), or d(TTGG), or approximately 0.5 μmol of d(CTGGCTCA), with a solution containing an equimolar amount of **1** or **2** in 1.5 mL of 0.1 M $NaClO_4$ at 20 ± 0.2 °C. The pH value was adjusted to 4.4 by addition of $HClO_4$. At this pH, guanine is not protonated at N7 (pK_a = 2.4 in 5′-GMP[24]) and the aqua ligands of **1** and **2** are in the OH_2 form (**1**, $pK_{a_1} = 5.37$, $pK_{a_2} = 7.21$;[25] **2**, pK_a = 6.0[26]).[27] During the reaction, pH was kept at 4.4 by addition of 0.5 μL portions of 2.3×10^{-3} M $HClO_4$ in 0.1 M $NaClO_4$, using a pHstat (Metrohm, Roucaire, Vélizy-Villacoublay, France). A 0.1 M $LiClO_4$ solution was used as the internal electrolyte instead of the standard KCl solution, in order to avoid diffusion of chloride into the reaction medium. Aliquots were withdrawn at several time intervals and quenched by addition of saturated KCl or KBr solution (depending on HPLC separation), and, after allowing 1.75 min for the exchange of the aqua ligands with halide, by cooling to liquid nitrogen temperature.[18]

HPLC Analysis. The reactions were analyzed by HPLC. The two systems which were employed for the analysis of the quenched aliquots consisted of (i) a Spectra Physics SP 8800 pump, coupled to a Spectra 100 UV detector, and a Chromjet integrator (Spectra Physics, Thermo Separation Products, Les Ulis, France) and (ii) two Shimadzu LC-6A pumps, coupled to an SPD-6A UV detector, and a C-R3A integrator (Shimadzu, Touzart & Matignon, Vitry-sur-Seine, France); both systems were connected to a Rheodyne 7125 valve with a 20 μL sample loop.

The chromatographic conditions were optimized for each reaction on a Kromasil C18 or Nucleosil C18 (250 × 4.6 mm i.d., 5 μm, 100 Å) column (Colochrom, Gagny, France). Operating conditions are described in the figure captions. The stability of the chloride- and bromide-quenched intermediates under the elution conditions was checked. Relative concentrations were determined from the ratio of the peak areas. The detection wavelength was 255 nm, chosen to be close to the quasi-isosbestic point of the reactions. The absorbance of the reaction mixture with **1** decreased during the reaction by 7% [d(GG)], 4% [d(TGG)], and 5% [d(TTGG)], respectively; this decrease correlated roughly with the formation of the chelate **C**. The chelate concentration, based on peak integration, was therefore corrected by a factor of 1.07, 1.04, and 1.05, respectively. No correction was applied for d(CTGGCTCA), since the absorbance did not vary appreciably in this case. The absorbance variations were negligible for the reactions with **2**.

Identification of the Reaction Intermediates. Identification of the monoadducts, trapped as chloro or bromo derivatives, was achieved by enzymatic digestion using the 3′-exonuclease venum phosphodiesterase (VPD) as described previously.[28] For d(GG), d(TGG), d(TTGG), and d(CTGG) (used as control; *vide infra*) reacting with **1**, the test is straightforward: only the 5′-monoadduct is digested and disappears from a subsequently recorded chromatogram of the VPD-treated mixture, since the digestion is inhibited at the 3′ side of a platinated base.[29−31] A 25 μL portion of the reaction mixture (10^{-3} M), quenched in 25 μL of saturated solution of KCl, was incubated

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with 2 μL of VPD (one enzymatic unit in 0.5 mL)[32] in 65 μL of a solution containing 3×10^{-2} M Tris−HCl, 3×10^{-2} M NaCl, and 4.5×10^{-3} M $MgCl_2$, at pH 8.5 and 37°C. For d(CTGGCTCA), the monoadducts (in the bromo form) were collected at the outlet of the column and digested by 25 μL of VPD without addition of buffer at room temperature, and the digested fragments were identified by coelution with a reference solution. The latter contained the mixture of products from the reaction of d(CTGG) with **1**, quenched with potassium halide at a time corresponding to the maximum concentration of the monoadducts and digested by VPD. The chromatogram of this control solution consisted therefore, apart from the peaks due to mononucleotides, of three peaks, identified as described above, as d(CTG*), d(CTGG*), and d(CTG*G*) (G* designates a platinum-bound guanine). The digestion of the major monoadduct from the platination of d(CTGGCTCA) yielded, apart from mononucleotides, a single product coeluting with d(CTG*). The major monoadduct was therefore identified as d(CTG*GCTCA), the 5′-platinated compound. The adducts from the platination of d(CTGGCTCA) by **2** were assigned using a similar procedure. In this case, the major monoadduct is also platinated at the 5′-G.

Determination of the Rate Constants. The rate constants for the reaction between the oligonucleotides and **1** ($k_{3'}$, $k_{5'}$, $k_{3'c}$, and $k_{5'c}$) (Figure 1) were calculated by an iterative program numerically integrating the differential kinetic equations and optimizing the fit between experimental and calculated concentrations curves. The minimized function was

$$f = \sum_{i=1}^{4}\{\sum_{k=1}^{n} w_{ik}[C_{ik}(\text{calc}) - C_{ik}(\text{exp})]^2\}^2$$

$$w_{ik} = \frac{1}{c_{ik}(\text{exp})}$$

where C_{ik} are the concentrations of the ith product at the time point k. Weighting with $1/c_{ik}$(exp) and summing up the squares of the sums over the individual curves prevent those having smaller values throughout from being fitted less well than the others.

Optimizations of the experimental curves for the reactions of the monofunctionally binding complex **2** were done using the program ITERAT.[33] ITERAT sums all the square deviations in a single loop but uses $1/\{c_{ik}(\text{exp})\}^2$ as weights w_{ik}.

Results

Methodology. Our method of kinetic analysis, previously outlined,[18] involves three key elements: (i) efficient quenching of the reaction mixture which has to last for at least 1 week (time needed to analyze all the samples); (ii) stability of the trapped monoadducts and well-resolved separation of the reaction products in HPLC; (iii) numerical evaluation of the rate constants. The chromatograms shown in Figure 2 demonstrate that, for each reaction, we were able to develop HPLC conditions allowing an excellent separation of the products and hence an accurate concentration measurement. As can be seen in the caption of Figure 2, the separation conditions may differ considerably from one reaction mixture to another. It is noteworthy that no common trend is apparent in the elution pattern of the four oligonucleotides and their adducts; the elution order of the species **N**, **I5′**, **I3′**, and eventually **C** is influenced by the chromatographic conditions and is different for each system. Thus, identification of the chromatographic peaks cannot be based on the order of elution but requires enzymatic digestion experiments.

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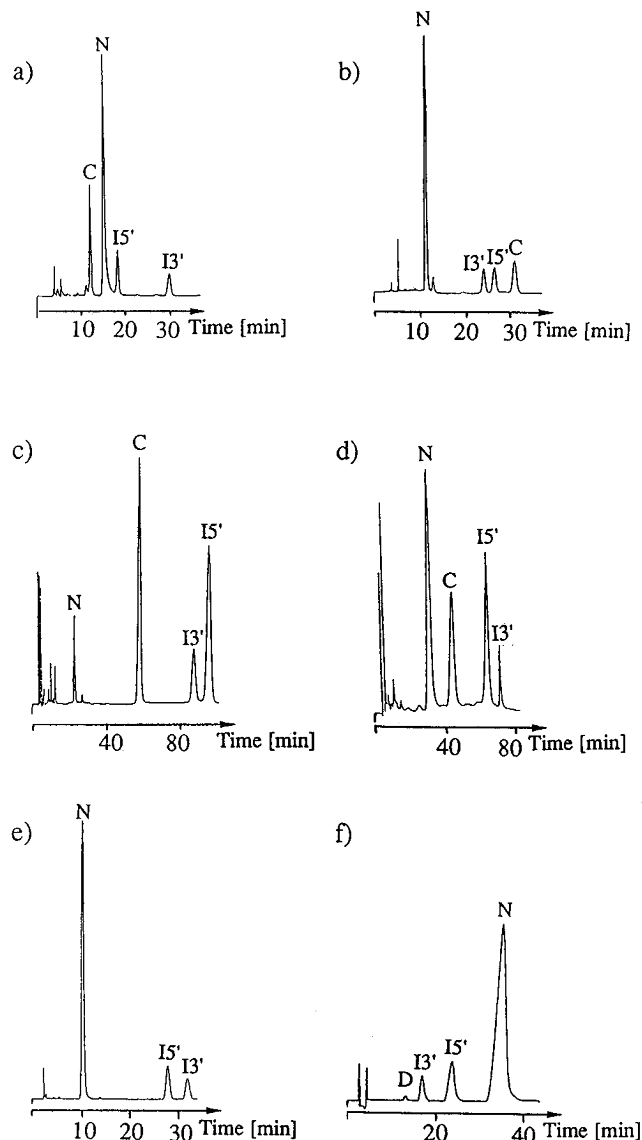

Figure 2. Typical chromatograms for approximately stoichiometric reaction mixtures quenched after 12 min by addition of an excess of KCl (a, b, c, e) or KBr (d, f). Reaction conditions: 0.1 M $NaClO_4$, pH = 4.4, T = 20 °C. (a) d(GG) + **1**, Spectra Physics chromatograph with a Nucleosil C18 column. Operating conditions: mobile phase, ammonium acetate buffer 0.01 M, pH = 4.7—acetonitrile (96.6:3.4 v/v during 16 min, then 95.2:4.8 v/v at 17 min); flow rate 0.8 mL min^{-1}; T = 27 °C. (b) d(TGG) + **1**, Spectra Physics chromatograph with a Nucleosil C18 column. Operating conditions: mobile phase, ammonium acetate buffer 0.01 M, pH = 4.7—acetonitrile (95.5:4.5 v/v); flow rate 0.8 mL min^{-1}; T = 25 °C. (c) d(TTGG) + **1**, Shimadzu chromatograph with a Kromasil C18 column. Operating conditions: mobile phase, ammonium acetate buffer 0.01 M, pH = 4.7—acetonitrile (94:6 v/v); flow rate 0.9 mL min^{-1}; T = 30 °C. (d) d(CTGGCTCA) + **1**, Shimadzu chromatograph with a Kromasil C18 column. Operating conditions: mobile phase, potassium chloride 1.5 M, ammonium acetate buffer 0.01 M, pH = 4.7 (gradient: 6% acetonitrile for 40 min; from 40 to 60 min, 6−7% acetonitrile); flow rate 0.8 mL min^{-1}; T = 41 °C. (e) d(TGG) + **2**, Shimadzu chromatograph with a Nucleosil C18 column. Operating conditions: mobile phase, ammonium acetate buffer 10^{-2} M, pH = 6.1—methanol (88:12 v/v); flow rate 0.8 mL min^{-1}; T = 27 °C. (f) d(CTGGCTCA) + **2**, Shimadzu chromatograph with a Kromasil C18 column. Operating conditions: mobile phase, potassium bromide 1 M, ammonium acetate buffer 0.01 M, pH = 4.7—acetonitrile (94:6 v/v); flow rate 0.8 mL min^{-1}; T = 41 °C.

Figure 3 shows a typical fit between the experimental and calculated concentrations. The numerical integration used for the simulation of the experimental concentration curves makes it possible to work in non-first-order conditions. We were

Table 1. Optimized Rate Constants for the Reactions of Oligonucleotides with *cis*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ and $[Pt(NH_3)_3(H_2O)]^{2+}$ at pH 4.4 and 20 °C in 0.1 M $NaClO_4$[a]

oligonucleotide	no. of expts	$k_{5'}$ (M^{-1} s^{-1})	$k_{3'}$ (M^{-1} s^{-1})	$k_{5'}/k_{3'}$	$10^3k_{5'c}$ (s^{-1})	$10^3k_{3'c}$ (s^{-1})	$k_{3'c}/k_{5'c}$
			cis-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ (**1**)				
d(GG)	5	0.39(3)	0.41(2)	**1.0**	1.5(1)	2.1(2)	**1.4**
d(TGG)	5	0.87(1)	0.87(6)	**1.0**	1.2(1)	1.7(1)	**1.4**
d(TTGG)	3	2.1(1)	1.7(2)	**1.2**	1.3(1)	3.9(7)	**3.0**
d(CTGGCTCA)	4	4.2(6)	2.0(2)	**2.2**	1.0(3)	3.3(4)	**3.3**
			$[Pt(NH_3)_3(H_2O)]^{2+}$ (**2**)				
d(TGG)	3	0.13(1)	0.09(1)	**1.5**			
d(CTGGCTCA)	4	1.1(1)	0.49(5)	**2.2**			

[a] Labeling of the rate constants is defined in Figure 1. Esd's are given in parentheses.

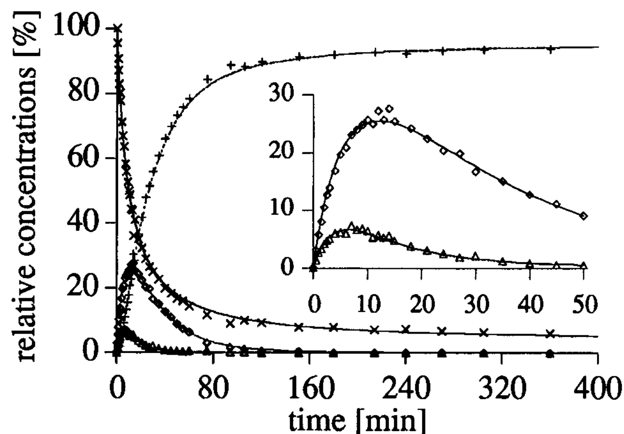

Figure 3. Calculated and experimental concentrations for the reaction between d(CTGGCTCA) and **1**: (×) unreacted oligonucleotide (**N**); (△) 3′-monoadduct (**I3′**); (◇) 5′-monoadduct (**I5′**); (+) chelate (C).

therefore able to work with stoichiometric amounts of reactants. With an excess of platinum, the first platination step would have been too fast to be accurately monitored and multiple platinations might have interfered.[23]

The chromatogram shown in Figure 2f corresponding to the reaction of **2** with d(CTGGCTCA) features in addition to the peaks due to the oligonucleotide (N) and the two monoadducts (I5′ and I3′) a fourth peak labeled D. This peak was due to a stable product, whose final concentration increased with an increase in the excess of **2**. If **2** was reacted with the octanucleotide in a molar ratio of 1.5 or more, **I5′** and **I3′** first increased and then decreased to give **D** as the major product. **D** was unambiguously identifed as the doubly-platinated adduct with $[Pt(NH_3)_3]^{2+}$ coordinated to both adjacent guanines. The identification was achieved by enzymatic digestion, gel electrophoresis, and mass spectrometry (MALDI-TOF). The kinetic scheme was therefore modified to take into account the formation of the doubly platinated species **D** from **I5′** and **I3′**. The detailed study of this reaction will be reported elsewhere.[23] For the reactions of the shorter oligonucleotides with **1** and **2**, no HPLC peaks were observed which could have been assigned to doubly-platinated species.

Reaction Kinetics. We have evaluated the rate constants for both platination ($k_{5'}$, $k_{3'}$) and chelation ($k_{5'c}$, $k_{3'c}$) steps of the reaction between complex **1** and single-stranded oligonucleotides containing a GG sequence. Complex **2** forms only monoadducts. Reactions with this complex were included in our study in order to investigate the influence of the platinum ligands on the kinetics of the platination step.

The rate constants are listed in Table 1. For both complexes, the platination rate constants $k_{3'}$ (platination of the 3′-guanine) and $k_{5'}$ (platination of the 5′-guanine) have similar values, increasing monotonically as the chain length increases. The constant $k_{5'}$ increases somewhat more rapidly, having twice the value of $k_{3'}$ in the case of the octanucleotide. For the chelation rate constants, the order is inverse. The ratio of the chelation rate constants $k_{3'c}/k_{5'c}$ increases with chain length from 1.4 for d(GG) to 3.3 for d(CTGGCTCA).

Discussion

Our long-term goal is to design metal complexes that would bind specifically to chosen sequences of DNA. To this end, we want to identify the factors which control the selective binding of cisplatin to GG sequences of DNA. In the present work, which is the first part of our kinetic study, we have investigated a series of single-stranded d(GpG)-containing oligonucleotides reacting with *cis*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ (**1**), the doubly-hydrolyzed form of cisplatin, and its triammine analog $[Pt(NH_3)_3(H_2O)]^{2+}$ (**2**), forming only monoadducts. In the second part, we will report the results of the reactions of a duplex oligonucleotide with the same platinum complexes. In the third part, the reactions of the monocationic forms of cisplatin, *cis*-$[PtCl(NH_3)_2(H_2O)]^+$ and *cis*-$[Pt(OH)(NH_3)_2(H_2O)]^+$, which are likely to be the two principal reactive species *in vivo*,[34] will be studied with single- and double-stranded oligonucleotides.

We are using an HPLC-based method allowing the determination of the platination and chelation rate constants for the two guanines separately.[18] The present study is intended to answer the following questions: (i) How does the oligonucleotide length influence the magnitude of the platination and chelation rate constants? (ii) How does the nature of the platinum ligands influence these rate constants? (iii) What are the lifetimes of the monoadducts formed by **1**?

First Step: Platination. Platination of the negatively charged oligonucleotides by the positively charged aqua complexes **1** and **2** is expected to proceed in two phases, a rapid formation of an outer-sphere complex and a rate-determining substitution reaction,[35] which is irreversible in our case. For instance, for complex **1** reacting with the 5′-G of the oligonucleotide **N** to form the monoadduct **I5′**, we have (**N···1**=outer-sphere complex formed by **1** and **N**)

$$\mathbf{N} + \mathbf{1} \underset{}{\overset{K_a}{\rightleftharpoons}} \mathbf{N{\cdots}1} \xrightarrow{k_{s5'}} \mathbf{I5'}$$

Under our experimental conditions, we observe an overall single step **N** + **1** → **I5′**. Since we cannot measure the concentration of the outer-sphere complex, [**N···1**], we do not know the net concentration of the free platinum complex, [**1**], either. On the other hand, we can determine the concentration of the total unreacted platinum complex, $[\mathbf{1_{un}}] = [\mathbf{1}] + [\mathbf{N{\cdots}1}]$, as the concentration of **1** at time = 0 minus the concentrations of all the platinated species: $[\mathbf{1_{un}}] = [\mathbf{1}]_0 - [\mathbf{I5'}] - [\mathbf{I3'}] - [\mathbf{C}]$. The

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rate of formation of **I5′** is

$$d[\mathbf{I5'}]/dt = k_{s5'}[\mathbf{N}\cdots\mathbf{1}] = k_{s5'}K_a[\mathbf{1_{un}}][\mathbf{N}]/(1 + K_a[\mathbf{N}])$$

$$K_a = \frac{[\mathbf{N}\cdots\mathbf{1}]}{[\mathbf{N}][\mathbf{1}]}$$

The value of the association equilibrium constant K_a is *a priori* unknown. For aqua ions of Mg^{2+} interacting with poly(dG-dC)$_2$, poly(A)-poly(U), poly(I)-poly(C), and poly(G)-poly(C), at pH = 7, T = 20 − 25 °C, and ionic strength = 0.1 M, a K_a value of 1000−2000 M^{-1} has been determined.[36,37] On the other hand, for the interaction between $[Ru(tpy)(bpy)O]^{2+}$ (tpy = 2,2′,2″-terpyridine, bpy = 2,2′-bipyridine) and the mononucleotide dianions CMP^{2-}, $dCMP^{2-}$, AMP^{2-}, and $dAMP^{2-}$, association constants between 15 and 48 M^{-1} have been determined.[38] For our platinum complexes forming outer-sphere complexes with di- to octanucleotides, we expect therefore K_a values of the order of $10-10^2$ M^{-1}. Thus, under our experimental conditions, $K_a[\mathbf{N}] \ll 1$ and $d[\mathbf{I5'}]/dt = k_{s5'}K_a[\mathbf{1_{un}}][\mathbf{N}]$. The platination rate constants listed in Table 1 are therefore equal to the product of the appropriate substitution rate constant $k_{s5'}$ or $k_{s3'}$ with the corresponding association equilibrium constant K_a.

According to Table 1, the platination rate constants increase monotonically with the oligonucleotide length. Going from d(GG) to d(TGG), the platination rate constants for both guanines increase by the same factor and the chelation rate constants remain roughly unchanged; this indicates that the presence of a 5′-phosphodiester group does not influence significantly the platination and the chelation of a d(GG) sequence in a single-stranded oligonucleotide. This is unexpected, since it has been suggested, on the basis of molecular mechanics calculations, that the 5′-phosphodiester group is capable of forming hydrogen bonds with the ligands of platinum in the pentacoordinated intermediates of the platination[39] and chelation[40] reactions. This result suggests that the increase of the observed platination rate constants with the chain length reflects mainly an increase of the association constant K_a, rather than variations of the substitution rate k_s. Elmroth and Lippard came to a similar conclusion from a comparison of pseudo-first-order rate constants for the reactions of *cis*-$[PtCl(NH_3)(C_6H_{11}NH_2)(H_2O)]^+$ with single-stranded oligonucleotides containing a d(GpG) sequence, on one hand, and a phosphorothioate group, on the other hand.[41]

Pullman et al.[42] investigated the influence of neighboring residues on the electrostatic potential at the different atomic positions of a base within double-stranded B-DNA. According to these calculations, the N7 atom in a guanine having a 5′-T and a 3′-G as neighbors (such as the 5′-guanine in d(CTGGCTCA)) experiences a more negative potential than that of a guanine having 5′-G and 3′-C as neighbors (which applies to the 3′-guanine in d(CTGGCTCA)). More generally, in a GG sequence, the negative potential at N7 is more enhanced on the 5′-G by its 3′ neighbor than *vice versa*. This agrees with recent experimental and theoretical results[43] suggesting that (i) guanines in GG sequences are more readily oxidized than other guanines and (ii) the 5′-guanine of a GG sequence is more reactive than the 3′-guanine. Although all these results[42,43] concern double-stranded DNA and cannot strictly apply to our single-stranded octanucleotide, it is possible that the slightly higher platination rate constants measured for the 5′-G of d(CTGGCTCA), compared to those for the 3′-G, reflect different electrostatic potentials at the two N7 atoms.

The diaqua complex **1** shows platination rate constants that are a factor of 4−10 larger than those for **2** throughout. This could be, in principle, due to four effects: (1) the platinum atom is expected to be less electrophilic in **2** than in **1**, since NH_3 is a better electron donor than H_2O; (2) the *cis* effects of NH_3 and H_2O may differently influence the reactivity of the leaving aqua ligand; (3) hydrogen-bonding with the O6 atom of the reacting guanine or that of the adjacent 3′- or 5′-guanine[39] could stabilize the pentacoordinated intermediate of the substitution reaction; this bonding should be stronger with H_2O than with NH_3;[44] (4) the association constants K_a might be larger for **1** than for **2**. Point 4 seems rather unlikely, since complexes **1** and **2** are very similar and there is no obvious reason that the stabilities of their outer-sphere complexes should be significantly different. A possible check of hypothesis 3 would consist of measuring the platination rate constants for **1** and **2** reacting with d(AA). Adenine possesses an NH_2 group at carbon C6, at variance with the carbonyl group of guanine, and thus, if $OH_2\cdots O6$ hydrogen bonding favors guanine platination by **1** over that by **2**, this effect would be absent in the case of adenine platination.

Second Step: Chelation. Comparison of the chelation rate constants measured for the 5′- and 3′-monoadducts of **1** with the different oligonucleotides (Table 1), shows that the cyclization on the 3′-monoadducts is always favored, and the ratio $k_{3'c}/k_{5'c}$ increases slightly with increasing oligonucleotide length. This can be rationalized by the fact that there is a tendency of the single-stranded oligonucleotides to assume B-DNA-like helical arrangements.[45] Molecular models of platinum monoadducts on a d(GG) segment of B-DNA show that the distance between the platinum atom and the N7 atom of the adjacent guanine is ∼4 Å in the 3′-monoadduct, whereas it is ∼5.5 Å in the 5′-monoadduct. Therefore, the helical arrangement favors chelation of the 3′-monoadduct and disfavors that of the 5′-monoadduct.[46] In very short oligonucleotides such as d(GG) or d(TGG), the mobility of the bases should overcome the structural predisposition favoring chelation of the 3′-monoadduct. Upon an increase in the oligonucleotide length, the helical structure becomes stabilized, which could explain the slight increase of the $k_{3'c}/k_{5'c}$ ratio. According to this reasoning, an even larger differentiation between the two chelation constants is expected for duplex oligonucleotides, and our preliminary results on d(TTGGCCAA)$_2$ reacting with **1** show that this is indeed the case. As suggested by a reviewer, the similar ratio by which the 5′-monoadducts are formed more rapidly and chelated (in the case of **1**) more slowly than the 3′-monoadducts might simply reflect the inherently greater reactivity of the 5′-G compared to the 3′-G. In support of this argument is our recent observation that when the monoadducts of the octanucleotide d(CTGGCTCA) with **2** react with a second equivalent of **2**, it is again the 5′-guanine which is platinated faster; i.e., the 3′-monoadduct forms the doubly-platinated complex more readily

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than the 5′-monoadduct.[23] However, similar comparisons in the case of the platination of the d(TTGGCCAA)$_2$ duplex show that, for two adjacent guanines, intrinsic nucleophilicity alone cannot explain both intra- and interstrand relative reactivities.[47]

Accumulation of the Monoadducts. As apparent from Figure 3, the combined effect of the slightly faster platination of the 5′-guanine and the slightly slower chelation of the 5′-monoadduct leads to different concentration profiles for the two monoadducts. The 5′-monoadduct accumulates in a significantly higher concentration and lasts longer than the 3′-monoadduct. Our preliminary results on the platination of d(TTGGCCAA)$_2$ with **1** show that this difference in monoadduct accumulation is even more pronounced in the case of the duplex.

Conclusion

For a series of single-stranded oligonucleotides containing the sequence d(GG), we have determined the rate constants for the reactions of the two individual guanines with *cis*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ (**1**) and $[Pt(NH_3)_3(H_2O)]^{2+}$ (**2**). For **1**, the rate constants for the conversion of the monoadducts into the chelate were measured as well. The two platination rate constants for all the oligonucleotides and both platinum complexes increase monotonically with increasing oligonucleotide length. For complex **1**, this increase can reach 1 order of magnitude from d(GG) to d(CTGGCTCA) in the case of the 5′-platination. This is consistent with an increase of the equilibrium constant K_a for the formation of an outer-sphere complex preceding the substitution reaction. As apparent from the different platination constants for **1** and **2** reacting with the same oligonucleotide, the platinum ligands have an influence on the platination kinetics; **1** reacts 4−5 times faster than **2**. The ratio of the two chelation rates $k_{3'c}/k_{5'c}$ increases with increasing oligonucleotide length from 1.4 to 3.3 upon going from the di- to the octanucleotide. We ascribe this effect to both a larger nucleophilicity of the 5′-guanine and a larger contribution of a single-helical conformation sterically favoring the cyclization of the 3′-monoadduct. Finally, we note that for reactions of **1**, the 5′-monoadduct reaches higher concentrations than the 3′-monoadduct and lasts longer, an observation which might be relevant to the formation of DNA−Pt−protein cross-links *in vivo*.

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