Triammineplatinum(II) Coordinated to a Guanine Does Not Prevent Platination of an Adjacent Guanine in Single-Stranded Oligonucleotides

Franziska Reeder, Jiří Kozelka,* and J. C. Chottard

Laboratoire de Chimie et Biochimie Pharmacologiques et Toxicologiques, URA 400 CNRS, 45, rue des Saints-Pères, 75270 Paris Cedex 06, France

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We have studied the reactions between the platinum aqua complex $[Pt(NH_3)_3(H_2O)]^{2+}$ and the oligonucleotides d(CTGGCTCA) and d(CTGG) in aqueous $NaClO_4$ solution. The oligonucleotides are expected to react with platinum mainly *via* the N7 atoms of the guanines,[1] and our aim was to measure and to compare the rates at which the two guanines of each oligonucleotide are platinated. Surprisingly, we have observed not only the formation of the two expected products with one of the two guanines bearing a $Pt(NH_3)_3^{2+}$ residue but also that of the doubly-platinated species, e.g., $[\{Pt(NH_3)_3\}_2\{d(CTGG\text{-}N7(3),N7(4)\}]$, and this even under stoichiometric conditions (Figure 1). Increasing concentrations of $NaClO_4$ favor the platination of the second guanine. These observations suggest that the negative charge of the oligonucleotide is effectively screened by the electrolyte and that the introduction of the cationic $Pt(NH_3)_3^{2+}$ unit is compensated for by migration of ions.

Methods and Results

The preparation of the oligonucleotides and the experimental methodology have been described previously.[2,3] $[PtCl(NH_3)_3]NO_3$ was prepared by an adaptation of the procedure described by Morita and Bailar.[4,5] $[Pt(NO_3)(NH_3)_3]NO_3$ was prepared by adding 0.99 equiv of $AgNO_3$ to a solution of $[PtCl(NH_3)_3]NO_3$. AgCl was removed by filtration after 24 h of stirring at room temperature. $[Pt(NO_3)(NH_3)_3]NO_3$ crystallized in vacuum over H_2SO_4 as colorless prisms. Yield: 80%. Anal. ($N_5H_9O_6Pt$) N, H, Pt Calcd: 52.7. Found: 51.7.

A 10^{-4} M solution of the oligonucleotide in 0.1 M $NaClO_4$ was reacted with varying amounts of $[Pt(NO_3)(NH_3)_3]NO_3$ in water. pH was 4.4 ± 0.1 during the reaction. In the periodically withdrawn aliquots, the reaction was stopped by additon of the same volume of saturated KBr solution and by cooling down to liquid nitrogen temperature, and the quenched mixtures were analyzed by HPLC, as previously described.[2,3] Figure 2 shows a typical chromatogram.[6] The chromatographic conditions are described in the figure caption. Identification of the peaks M5′ and M3′ as the 5′- and 3′-monoadducts was achieved by enzymatic digestion of the corresponding fractions using the exonuclease venum phosphodiesterase (VPD, Sigma).[2,3] Peak D was identified as the doubly platinated species by reacting the oligonucleotides with excess (≈50%) $[Pt(NH_3)_3(H_2O)]^{2+}$. The peaks due to the 5′- and 3′-monoadducts first increased and then decreased to give D as the major product. The formation of a diplatinated adduct was furthermore confirmed by means of mass spectrometry (MALDI−TOF−MS) and gel electrophoresis. The MALDI spectra have been performed at the Curie Institute on an in-house built MALDI specrometer with a linear TOF analyzer as described previously.[7] The negative-ion mass spectrum of the untreated octanucleotide is shown in Figure 3a. The intense peak at *m*/*z* 2384 corresponds to the deprotonated 8-mer [d(CTGGCTCA) − H]$^-$; a minor peak at *m*/*z* 2421 is due to substitution of protons by alkali ions on the

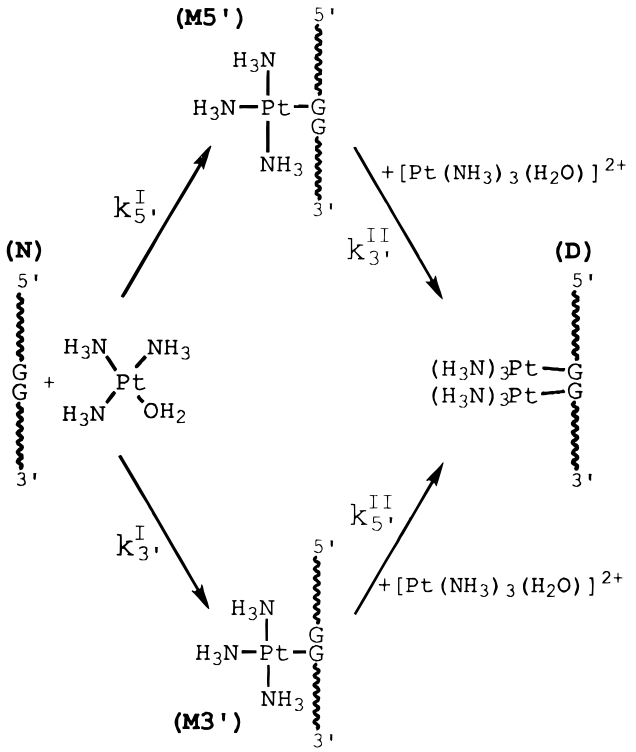

Figure 1. Observed reactions of $[Pt(NH_3)_3(H_2O)]^{2+}$ with GG-containing single-stranded oligonucleotides.

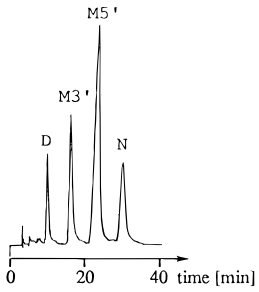

Figure 2. Typical chromatogram recorded at time = 240 min for a reaction of $[Pt(NH_3)_3(H_2O)]^{2+}$ with d(CTGGCTCA) (starting molar ratio: 1.1:1) in 0.1 M $NaClO_4$, pH = 4.4, T = 293 K, after quenching by addition of excess of KBr. Operating conditions: Kromasil C18 column, 250 × 4.6 mm ID, 5 μm, 100 Å (Colochrom, Gagny, France); mobile phase; KBr 1 M, ammonium acetate buffer 0.01M, pH = 4.7/acetonitrile 94:6 (v/v); flow rate 0.8 mL min^{-1}; T = 41 °C.

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(5) The modification consisted in neutralizing the solution of $[PtCl(NH_3)_3]NO_3$ with 0.5M HCl to pH ≈ 7 (instead of adding HCl in excess), and evaporating the solvent in vacuo over concentrated H_2SO_4. Three recrystallizations from a minimum of water at 4 °C yielded analytically pure $[PtCl(NH_3)_3]NO_3$. Yield: 42%. Anal. ($N_4H_9ClO_3Pt$): N, H, Cl, Pt.
(6) HPLC analysis of the reaction aliquots was performed on two Shimadzu LC-6A pumps coupled to a SPD-6A UV detector and a C-R3A integrator (Shimadzu, Touzart & Matignon, France). The system was connected to a Rheodyne 7125 valve with a 20 μL sample loop.
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Table 1. Optimized Rate Constants [M^{-1} s^{-1}][a–c]

oligonucleotide	no. of expts	electrolyte	$k^{I}_{5'}$ ↓ − 5′G p G3′−	$k^{II}_{5'}$ −Pt− ↓ − 5′G p G3′ −	$k^{I}_{5'}/k^{II}_{5'}$	$k^{I}_{3'}$ ↓ − 5′G p G3′−	$k^{II}_{3'}$ −Pt− ↓ − 5′G p G3′ −	$k^{I}_{3'}/k^{II}_{3'}$
d(CTGG)	1	10^{-1} M $NaClO_4$	0.70(1)	0.17(2)	4	0.35(1)	0.09(1)	3.9
d(CTGGCTCA)	4	10^{-1} M $NaClO_4$	1.1(1)	0.32(4)	3.4	0.49(5)	0.12(3)	4.1
	1	10^{-2} M $NaClO_4$	3.88(5)	0.83(5)	4.7	2.00(4)	0.31(2)	6.5
	1	10^{-3} M $NaClO_4$	4.37(3)	0.83(3)	5.3	2.27(3)	0.32(1)	7.0

[a] See Figure 1 for the reaction scheme. [b] T = 193 K, pH = 4.4. [c] Standard deviations of the nonlinear fitting procedure are given in parentheses. Where data from $n > 1$ experiments were available, the mean value is listed with $\sigma = (\sum_{i=1}^{n} (x_i - \bar{x})^2/(n-1))^{1/2}$ in parantheses.

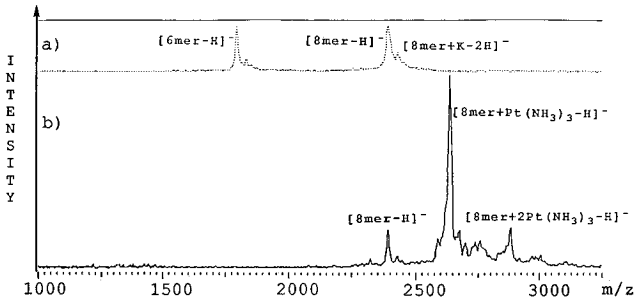

Figure 3. Negative-ion MALDI mass spectra: (a) d(CTGGCTCA) and d(GTTAAC) (as reference); (b) the reaction mixture of d(CTGGCTCA) and $[Pt(NH_3)_3(H_2O)]^{2+}$ at the end of the reaction. (Matrix: 80% anthranilic acid + 20% nicotinic acid; laser fluence: 60 mJ/cm^2.)

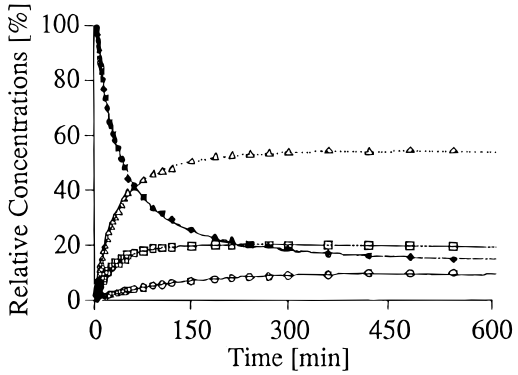

Figure 4. Calculated and experimental concentrations for the reaction between d(CTGGCTCA) and $[Pt(NH_3)_3(H_2O)]^{2+}$ in 0.1M $NaClO_4$. Key: (●) unreacted d(CTGGCTCA) (N); (□): 3′-monoadduct (M3′); (Δ): 5′-monoadduct (M5′); (○): diadduct (D). For this experiment, the optimized rate constants were as follows: $k^{I}_{5'} = 1.18$; $k^{II}_{5'} = 0.37$; $k^{I}_{3'} = 0.52$; $k^{II}_{3'} = 0.11$ M^{-1} s^{-1}.

phosphate groups $[d(CTGGCTCA) + K - 2H]^-$ (theor = 2422). No fragment ions have been detected. Figure 3b shows the spectrum of the reaction products of d(CTGGCTCA) with $[Pt(NH_3)_3(H_2O)]^{2+}$ at the end of the reaction (time > 20 h). The peaks at m/z = 2384, m/z = 2628 and m/z = 2876 were identified respectively as the unplatinated octanucleotide (theor = 2385.7), the monoadducts (theor = 2631.9) and the diadduct (theor = 2878.1).

The rate constants were determined by numerical integration of the appropriate differential equations and optimizing the fit between experimental and calculated concentrations, using the program ITERAT.[8] In Figure 4, the experimental and calculated concentrations are plotted vs time for one of the experiments. Table 1 lists the optimized rate constants.

Discussion

Nucleophilic substitution reactions between a positively charged metal aqua complex $M(H_2O)_n^{p+}$ and a negatively charged ligand L′ have been shown to proceed in two steps: i) the reversible formation of an outer-sphere complex, and ii) the actual replacement of a coordinating ligand from the inner-sphere (eq 1).[9]

$$M(H_2O)_n^{p+} + L'^{q-} \overset{K_a}{\rightleftharpoons} [M(H_2O)_n^{p+} \cdots L'^{q-}] \xrightarrow{k_s} \xrightarrow{k_{app}} M(H_2O)_{n-1}L'^{(p-q)+} + H_2O \quad (1)$$

The reactions studied in this work involve a nucleophilic substitution of the aqua ligand of $[Pt(NH_3)_3(H_2O)]^{2+}$ by the N7 atom of a guanine. Since the entering guanine is part of a negatively charged oligonucleotide and the electrophilic species is a positively charged aqua complex, such reactions are expected to follow the mechanism summarized in eq 1. The rate constants determined according to the kinetic scheme shown in Figure 1 correspond, supposed that eq 1 applies, to the apparent overall rate constants k_{app}. As we have argued previously[3], under our reaction conditions, the product of K_a (eq 1) and the oligonucleotide concentration is far below 1, and $k_{app} \cong k_s K_a$.

If a $Pt(NH_3)_3^{2+}$ group is already attached to a guanine, it is expected to repel a second $[Pt(NH_3)_3(H_2O)]^{2+}$ complex reacting with the adjacent base. Thus, both K_a and k_s for the second platination are expected to decrease, since both the stability of the outer-sphere complex and that of the transition state for the inner-sphere substitution (with a partly formed Pt−N7 bond) are enhanced by the attraction between the charges of the platinum entity and of the oligonucleotide, and this enhancement is expected to diminish. Surprisingly, as is apparent from Table 1, k_{app} diminishes, in 10^{-3}–10^{-1} M $NaClO_4$, by less than 1 order of magnitude, indicating that the repelling effect of the first $Pt(NH_3)_3^{2+}$ unit on K_a and k_s is only very moderate. We conclude that the binding of $Pt(NH_3)_3^{2+}$ to a guanine induces migration of cations away from the oligonucleotide chain and of anions in the opposite direction, compensating for the introduction of positive charge to the oligonucleotide.

The screening of DNA by counterions and its inhibitory effect on substitution reactions are well-known. For instance, Schaller et al. investigated the reactions between DNA and the two hydrolyzed forms of the antitumor drug cisplatin, *cis*-$[PtCl(NH_3)_2(H_2O)]^+$ and *cis*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$.[10] They have found that the reactions of both species were slowed down by increasing salt concentration, the effect being stronger in the case of *cis*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$. This has been interpreted as masking of the attractive electrostatic forces by the added salt. The apparent rate constants that we have measured for the

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reactions between d(CTGGCTCA) and $[Pt(NH_3)_3(H_2O)]^{2+}$ are also dependent on the ionic strength (Table 1). First, all rate constants increase when the electrolyte is diluted, and secondly, the ratio k^{I}/k^{II} increases for both the 5′ and 3′ guanines at reduced salt concentration, in accord with diminished migration of counterions in low-salt conditions.

Sequence-dependent metal−DNA interactions are receiving growing interest,[11] and reactions with oligonucleotides are frequently used to model such interactions. G_n tracts have been shown to be hotspots for the binding of several antitumor platinum complexes[12] and kinetic studies of this preferential binding are currently underway in different laboratories including ours.[2,3,13,14] The present communication is meant as a caveat for workers in this area to be aware of the possibility of cumulative binding of two metal centers to adjacent nucleobases. According to our results, such binding is likely especially in high-salt conditions. In cases where a chelating metal complex (e.g. *cis*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$) reacts with a GG sequence, the formation of the intrastrand G−M−G cross-link will, of course, compete with the second metal coordination. However, many authors use pseudo-first-order conditions with the metal complex in excess, and in this case the coordination of the second metal could become significant and jeopardize the kinetic measurement.

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