# Medium Effects on Reactivity Profiles for Platination of Phosphorothioate-Containing Oligonucleotides

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Reactions of *cis*-$[Pt(NH_3)(NH_2C_6H_{11})Cl(OH_2)]^+$ with d(Tp(S)T) and $d(T_np(S)T_{16-n})$, $n = 1$, 4, 8, 12, and 15, were investigated by use of HPLC in an aqueous medium with pH 4.1 ± 0.1 and sodium and magnesium ion concentrations varying between 1.5 mM and 0.50 M. Platination of the oligonucleotide fragments is favored over platination of d(Tp(S)T) in the whole salt concentration interval studied. The maximum rate enhancement after incorporation of the p(S)-site into the polymeric DNA environment is observed for $d(T_8p(S)T_8)$, which reacts up to ca. 500 times faster than d(Tp(S)T), after suitable changes of the cation concentrations in the reaction medium. The platination rates of the oligonucleotide fragments $d(T_np(S)T_{16-n})$ decrease with increasing salt concentration. For a given phosphorothioate position, the rate also decreases when the cations in the medium are changed from $Na^+$ or $K^+$ to $Mg^{2+}$, even at constant ionic strength. The reactions with embedded p(S)-sites in $d(T_np(S)T_{16-n})$, $n = 4$, 8, and 12, were found to be kinetically favored over reactions with the 5′- and 3′-end positions. In a reaction medium containing monovalent cations there is a strong preference for platination of $d(T_8p(S)T_8)$, whereas $d(T_4p(S)T_{12})$ and $d(T_{12}p(S)T_4)$ show intermediate reactivity compared with fragments with $n = 1$ and 15. In contrast, no kinetic discrimination is found between the p(S)-sites in $d(T_np(S)T_{16-n})$, $n = 4$, 8, and 12, in the presence of $Mg^{2+}$. The results are interpreted in terms of a general mechanism where preaccumulation of the cationic Pt(II) complex on the oligomers is required for product formation. The kinetics are consistent with a reaction model that includes release of cations from the DNA surface during the adduct formation process.

## Introduction

Reactions of transition metal complexes with biomolecules, such as nucleosides, mono- and polynucleotides, sulfur-containing amino acids, and DNA, are the subject of large current interest. One of the major objectives is to increase the understanding of the mechanism of action of anticancer active platinum complexes.[1,2] The generally accepted intracellular metabolic pathway comprises interaction with nuclear DNA and the preferential formation of an intrastrand cross-link between adjacent guanines.[3−13] The molecular mechanism for the reaction preceding the adduct formation step is still not fully understood, however. It is likely though that the polyelectrolyte properties of DNA play a crucial role for the intracellular distribution of currently used platinum drugs. The positively charged reactive platinum(II) metabolites[2a,14] are electrostatically attracted by the negatively charged phosphate groups along the DNA backbone. As a result, the local platinum concentration is likely to be higher in the close vicinity of the DNA than in the surrounding cytosol.[15−18] For concentration-dependent reactions, such accumulation should have a significant influence on the observed reactivity of any reactive group located on the DNA surface.

The ability of DNA to modulate transition metal reactivity has been observed in several studies. For example, the apparent rate for thermal electron transfer[19] and substitution reactions[20−30] of freely diffusing cationic metal complexes with various reactants has been found to increase in a polymeric DNA environment. For the latter type of reactions, the composition of the reaction medium and the detailed DNA environment have

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been shown to have a large influence on the observed rate constants.[22−32] Systematic studies of interactions between positively charged metal complexes of Pt(II), Pd(II), and Au(III) and target sites incorporated into short poly-d(T) oligomers have revealed a DNA-promoted effect in reactivity range spanning 6 orders of magnitude, when compared with reference reactions of monomers such as d(Tp(S)T), d(GpG), d($^{s4}$U), and d($^{s6}$I).[24,25,28−30,33] Further, the dependence of the observed rate constants on the concentration of neutral salts in the reaction medium, which has been observed for some of these systems, fits qualitatively well with a reaction mechanism in which electrostatic preaccumulation of the cationic reactants on the oligomer takes place prior to the rate-determining formation of the adduct.[25,28] However, a detailed mechanistic interpretation of the salt dependence is hampered by a lack of more precise knowledge concerning the extent and dynamics of associated cations around the used oligonucleotide fragments.[34]

Short-size DNA and oligonucleotides exhibit different physical properties compared with B-DNA. For example, the local concentration of cations in the condensation layer does not conform to the uniform level typical of extended DNA.[35−37] The effective concentration of an approaching cationic species is thus likely to depend on the location of the target site chosen for a specific interaction. A preliminary investigation of the kinetics for reactions of a metabolite, *cis*-[Pt(NH$_3$)(NH$_2$C$_6$H$_{11}$)Cl(OH$_2$)]$^+$, of an orally active Pt(IV) anticancer drug (e.g., JM-216)[38,39] and two phosphorothioate-containing oligonucleotides was reported recently.[28] At the pH used, accumulation of oxonium ions could be excluded as a contribution to the observed reactivity variations.[40−42] A significant kinetic preference was observed for adduct formation with the middle position in d(T$_8$p(S)T$_8$) compared with the 5′-end in d(Tp(S)T$_{15}$). The reactivity at the 5′-end was found to be higher compared with that of d(Tp(S)T), possibly reflecting an influence from nonuniform electrostatic accumulation of the platinum complex on the two oligomers studied. The aim of the present work has been to obtain more complete experimental data on how the nature and concentration of neutral salts in the reaction medium affect the apparent reactivity of a given phosphorothioate site. The kinetic influence associated with the location of the p(S)-site in a given size oligonucleotide fragment d(T$_n$p(S)T$_{16-n}$) was also investigated. Analysis of current experimental data as a function of the concentration of inert cations in the reaction medium supports a mechanism where adduct formation is accompanied by the release of cations from the DNA surface regardless of location of the binding site.

## Experimental Section

**Materials and Solutions.** Standard aqueous buffer solutions were prepared from potassium hydrogen phthalate (KHC$_8$H$_4$O$_4$, ACROS), and the pH was adjusted by addition of HClO$_4$ (Merck, p.a.).[43] The phthalate buffer was diluted 10 or 100 times for the kinetics experiments, and the pH was determined by use of a standard pH electrode (Orion). NaClO$_4$ (Merck, p.a.) or Mg(ClO$_4$)$_2$ (ACROS, p.a.) was added to adjust the ionic strength. Stock solutions were kept at room temperature. Water was doubly distilled from quartz.

The compound *cis*-[Pt(NH$_3$)(NH$_2$C$_6$H$_{11}$)Cl$_2$], **1**, was synthesized according to literature.[44] The complex *cis*-[Pt(NH$_3$)(NH$_2$C$_6$H$_{11}$)ClX]$^{+/0}$, X = DMF or NO$_3^-$, was prepared by addition of 0.98 equiv of AgNO$_3$ (Baker Analyzed Reagent) to **1** dissolved in DMF (LAB-SCAN). The reaction mixture was allowed to vortex in the dark for ca. 20 h, and the AgCl precipitate was removed by centrifugation. Platinum(II) stock solutions were stored in the dark at 8 °C. Addition of *cis*-[Pt(NH$_3$)(NH$_2$C$_6$H$_{11}$)ClX]$^{+/0}$, X = DMF or NO$_3^-$, to an aqueous solution results in rapid and quantitative conversion to the corresponding aqua complex *cis*-[Pt(NH$_3$)(NH$_2$C$_6$H$_{11}$)Cl(OH$_2$)]$^+$, **2**.

Aqueous stock solutions of the phosphorothioates d(Tp(S)T) (KEBO-lab), d(Tp(S)T$_{15}$), d(T$_4$p(S)T$_{12}$), d(T$_8$p(S)T$_8$), d(T$_{12}$p(S)T$_4$), and d(T$_{15}$p(S)T) (Scandinavian Gene Synthesis AB) were kept frozen at −20 °C. Concentrations were determined spectrophotometrically at 260 nm using calculated extinction coefficients for d(T$_{16}$) and d(T$_2$),[45,46] $\epsilon_{260}$ = 130 200 and 16 800 M$^{-1}$ cm$^{-1}$, respectively. Spectra were recorded at ambient temperature and pressure using a Milton Roy 3000 diode-array spectrophotometer and thermostated 1.00 cm Quartz Suprasil cells.

**HPLC Measurements.** The platination reaction was monitored by use of a LaChrom chromatograph (Merck-Hitachi, working under Microsoft Windows 3.51) with a D-7000 interface and a D-7400 UV/vis detector set at 260 nm. Unplatinated phosphorothioates were separated from platinated products by use of a reverse phase Protein & Peptide C18 (100 × 4.6 mm i.d., 10 $\mu$m particle diameter) column (Vydac) equipped with a guard, or a Poros R2/H (100 × 4.6 mm i.d., 10 $\mu$m particle diameter) column (PerSeptive Biosystems). The columns were kept at 25.0 ± 0.2 °C by use of an L-7350 oven and a cooling module (Merck-Hitachi). Solutions of 0.10 M ammonium acetate (NH$_4$-OAc, Merck), **A**, adjusted to pH 6.0 with acetic acid (HOAc, Merck), and a 1:1 mixture of **A** and acetonitrile (CH$_3$CN, LAB−SCAN), **B**, were used as eluents for the Vydac column. A low-pressure gradient was used at a constant flow of 1 mL/min. A linear gradient in which the ratio **A**:**B** changed from 100:0 to 64:36 over a period of 30 min was used to separate products from reactants. In the case of the Poros R2/H column, solutions of 0.1% TEAA (triethylammoniumacetate, Merck) in H$_2$O, **C**, and 0.1% of TEAA in CH$_3$CN (LAB-SCAN), **D**,

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were used as eluents. A constant ratio of **C:D** of 96:4 for 2 min was followed by a linear gradient in which the ratio **C**:**D** changed from 96:4 to 87:13 over a period of 9 min, at a constant flow of 3 mL/min, was used for separation. The chromatograms were evaluated by use of an on-line HPLC System Manager Software. The time-dependent changes of integrated peak areas for reactants, as a sum of the $R_p$ and $S_p$ diastereomers,[25,47] and corresponding products were used for the kinetics evaluation.

**Kinetics.** Reactions between *cis*-$[Pt(NH_3)(NH_2C_6H_{11})Cl(OH_2)]^+$, **2**, and the oligonucleotides d(Tp(S)T), d(Tp(S)$T_{15}$), d($T_4$p(S)$T_{12}$), d($T_8$p(S)$T_8$), d($T_{12}$p(S)$T_4$), and d($T_{15}$p(S)T) were studied at pH 4.1 ± 0.1. At this pH, the distribution between **2** and its corresponding hydroxo complex is rather insensitive to possible local pH differences between the bulk solution and the oligonucleotide surface.[40−42] A dilute phthalate buffer was used to minimize the influence from competitive reactions of the metal with buffer anions.[48] The reactions were studied as a function of the concentrations of mono- and divalent cations in the medium in the interval 1.5 mM ≤ [cation] ≤ 0.50 M. The cation concentration, defined as [cation] = ($[Na^+]$ + $[K^+]$) or ($[Mg^{2+}]$ + $[K^+]$), was varied by addition of $NaClO_4$ or $Mg(ClO_4)_2$ to the buffers. The potassium ion concentration was 5.0 mM at [cation] = 35 mM and 0.5 mM at other concentrations. Metalation of the phosphorothioates was studied under pseudo-first-order conditions, with at least a 10-fold excess of the monoaqua platinum(II) complex, **2**. Typical reaction conditions were $C_{Pt} = (0.3-3) \times 10^{-4}$ M and [oligonucleotide] = $(0.4-2) \times 10^{-5}$ M. Reactions were initiated by addition of a small volume of **2** in DMF to a buffered and thermostated solution of oligonucleotide. Sample aliquots were withdrawn at different time intervals during the course of the reaction, and they were immediately quenched by dilution in buffer followed by freezing in liquid nitrogen. Samples were stored in liquid nitrogen at −196 °C and analyzed by HPLC within 48 h after sampling, directly after thawing. Observed pseudo-first-order rate constants for the platination reactions were determined from a fit of single exponentials to the time-dependent decrease of the peak area associated with unreacted oligonucleotide. Errors correspond to one standard deviation.

## Results

**Kinetics Evaluation.** Addition of **2** to a solution containing a mixture of the $R_p$ and $S_p$ diastereomers of phosphorothioate-containing oligonucleotides results in a decrease of the peak areas eluting with a retention time typical for that of unreacted starting material and an increase of peaks originating from products with longer retention times (chromatograms in Figure S1). The time course for the decrease of unreacted oligonucleotide peaks obeys pseudo-first-order kinetics. Typical plots of experimental data for reactions of d($T_8$p(S)$T_8$), d($T_{12}$p(S)$T_4$), and d(Tp(S)$T_{15}$) with **2** are shown in Figure 1, together with the corresponding best fit of single exponentials to the experimental data. There is a linear relationship with insignificant intercept between the observed rate constants, $k_{obsd}$, and the total concentration of Pt(II), $C_{Pt}$, or $[Pt(II)]_{tot}$ (experimental data in Table S1), in agreement with previous studies on related systems.[24,25,28] Apparent second-order rate constants could thus be calculated according to $k_{2,app} = k_{obsd}/[Pt(II)]_{tot}$.

**Platination of d(Tp(S)T).** The reactions between d(Tp(S)T) and **2** were monitored to determine the inherent reactivity of the phosphorothioate group and its dependence on the ionic charge and concentration of the cations in the medium. For cation concentrations > 10 mM, there is little variation with the ionic charge and concentration, the average second-order rate constant being ca. 0.4 $M^{-1}$ $s^{-1}$, cf. Table 1 and Figure 2a. At the lowest salt concentration used, 1.5 mM, the platination rate is slightly higher in the presence of monovalent cations than in the presence of $Mg^{2+}$, $k_{2,app}$ being 1.0 ± 0.5 and 0.5 ± 0.1 $M^{-1}$ $s^{-1}$, respectively (experimental data in Tables S2 and S3).

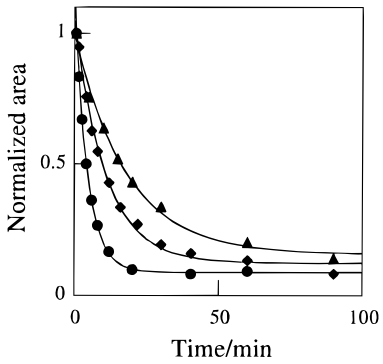


**Figure 1.** Normalized integrated HPLC peak areas vs time for the reactions of **2** at 25.0 °C, pH 4.1 ± 0.1, [cation] = 0.035 M, [Pt(II)] = $(5.00-5.04) \times 10^{-5}$ M, [oligonucleotide] = $(4.99-5.02) \times 10^{-6}$ M with d(Tp(S)$T_{15}$) (▲), d($T_8$p(S)$T_8$) (●), and d($T_{12}$p(S)$T_4$) (◆).

**Table 1.** Selected Second-order Rate Constants for the Reaction of **2** with d(Tp(S)T) and d($T_n$p(S)$T_{16-n}$) as a Function of Type and Concentration of Cation in the Supporting Electrolyte

| oligonucleotide | predominant cation[a] | $k_{2,app}/M^{-1}$ $s^{-1}$ for [cation]/mM = 1.5 | 35 | 200−500 |
|---|---|---|---|---|
| d(Tp(S)T) | $Na^+$ | 1.0 ± 0.5 | 0.4 ± 0.1 | 0.5 ± 0.1[b] |
| | $Mg^{2+}$ | 0.5 ± 0.1 | 0.3 ± 0.1 | 0.4 ± 0.2[b] |
| d(Tp(S)$T_{15}$) | $Na^+$ | 30 ± 10 | 19 ± 3 | 1.4 ± 0.7[b] |
| | $Mg^{2+}$ | 8.1 ± 1.0 | 1.7 ± 0.2 | 0.6 ± 0.2[b] |
| d($T_4$p(S)$T_{12}$) | $Na^+$ | | 49 ± 8 | 2.9 ± 0.9[c] |
| | $Mg^{2+}$ | | 3.4 ± 0.7 | 1.3 ± 0.5[c] |
| d($T_8$p(S)$T_8$) | $Na^+$ | 200 ± 40 | 74 ± 4 | 6.0 ± 2.0[b] |
| | $Mg^{2+}$ | 35 ± 6 | 3.6 ± 0.3 | 1.3 ± 0.3[b] |

[a] $[K^+]$ = 5 mM for [cation] = 35 mM, and 0.50 mM for all other [cation]. [b] Average of rate constants in the interval 0.20−0.50 M. [c] [cation] = 0.50 M.

**Salt Influence on the Platination Rate of Oligonucleotides; Reactions with d($T_8$p(S)$T_8$) and d(Tp(S)$T_{15}$).** The influence of the cations in the reaction medium on the rate of platination of the oligonucleotides d($T_8$p(S)$T_8$) and d(Tp(S)$T_{15}$) was studied for varying concentrations of monovalent ($Na^+$) and divalent ($Mg^{2+}$) counterions. The platination rate constants, $k_{2,app}$, are always larger in the presence of monovalent cations than in the presence of $Mg^{2+}$; see Table 1 and Figure 2b,c (experimental data in Tables S2 and S3). This tendency is most pronounced at cation concentrations of 1.5 mM and for the platination of d($T_8$p(S)$T_8$), the second-order rate constants being 200 ± 40 and 35 ± 6 $M^{-1}$ $s^{-1}$, in the presence of mono- and divalent bulk cations, respectively. Under identical reaction conditions, the corresponding rate constants for platination of d(Tp(S)$T_{15}$) were determined to be 30 ± 10 and 8.1 ± 1.0 $M^{-1}$ $s^{-1}$, respectively. Further, the platination rate decreases for increasing salt concentrations. The apparent rate constants exhibit a strong salt dependence up to a concentration of ca. 0.10 M. Above that value, the rate constants are approximately constant: $k_{2,app}$(d($T_8$p(S)$T_8$)) = 6 ± 2 and 1.3 ± 0.3 $M^{-1}$ $s^{-1}$ in the presence of mono- and divalent cations, respectively. The corresponding rate constants for platination of d(Tp(S)$T_{15}$) are 1.4 ± 0.7 and 0.6 ± 0.2 $M^{-1}$ $s^{-1}$, respectively.

**Reactivity Profiles for Platination of d($T_n$p(S)$T_{16-n}$); *n* = 1, 4, 8, 12, and 15.** Rates of platination of the phosphorothioate site in the oligonucleotide environment were studied as a function of the specific position along the phosphodiester backbone in d($T_n$p(S)$T_{16-n}$), *n* = 1, 4, 8, 12, and 15. The rate

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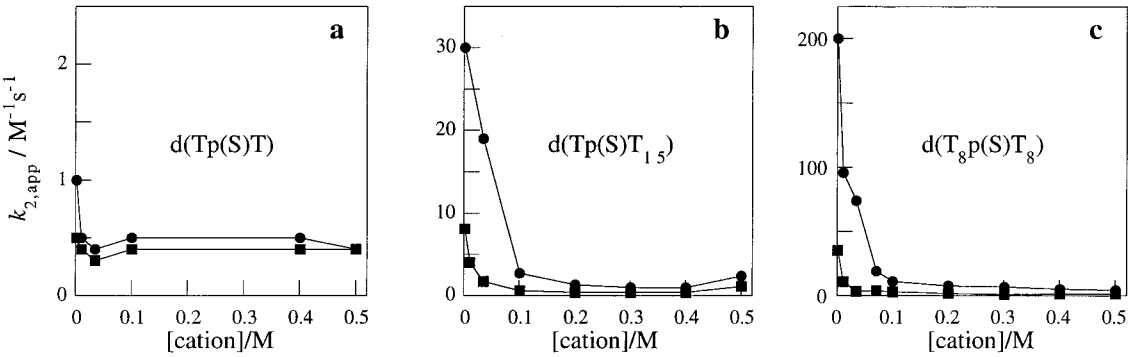


**Figure 2.** Plots of the apparent second-order rate constant vs cation concentration for the reaction of **2** at pH 4.1 ± 0.1 and 25.0 °C, [Pt(II)] = (3.78−27.6) × $10^{-5}$ M, [oligonucleotide] = (4.00−18.4) × $10^{-6}$ M, and variable concentration of $Na^+$ (●) and $Mg^{2+}$ (■), with (a) d(Tp(S)T), (b) d(Tp(S)$T_{15}$), and (c) d($T_8$p(S)$T_8$).

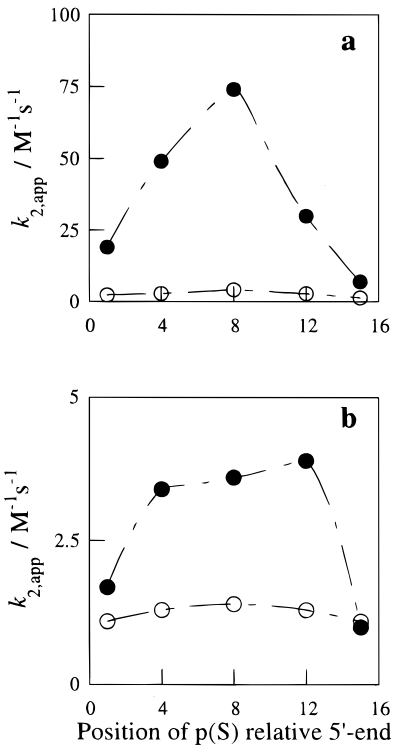


**Figure 3.** Reactivity profiles for the platination of the p(S) moiety as a function of its location along the oligonucleotide at 25.0 °C and pH 4.1 ± 0.1, [Pt(II)] = (5.00−5.04) × $10^{-5}$ M, [d($T_n$p(S)$T_{16-n}$)] = (4.99−5.03) × $10^{-6}$ M, $n$ = 1, 4, 8, 12, and 15, [cation] = 0.035 M (●), and [cation] = 0.50 M (○) in the presence of (a) $Na^+$ as predominant cation and (b) $Mg^{2+}$ as predominant cation.

was determined at two different salt concentrations, 35 mM and 0.50 M, with both mono- and divalent cations in the supporting electrolyte. During these experiments, the concentrations of **2** and the oligonucleotides were kept constant to enable comparison between experimental data, $C_{Pt}$ = (5.0 ± 0.1) × $10^{-5}$ M and [d($T_n$p(S)$T_{16-n}$)] = (5.0 ± 0.1) × $10^{-6}$ M (primary data in Tables S2 and S3). Reactivity profiles with the apparent second-order rate constants as a function of position of the phosphorothioate site relative to the 5′-OH group are shown in Figure 3. Selected rate constants are given in Table 1.

In the presence of monovalent cations, platination of the middle position is favored both at high and low cation concentrations with $k_{2,app}$ = 74 ± 4 and 4.1 ± 0.4 $M^{-1}$ $s^{-1}$ at [cation] = 0.035 and 0.50 M, respectively; see Figure 3a. The platination rate decreases gradually and to a similar extent as the 5′- and 3′-ends are approached. The decrease is slightly more pronounced on the 3′-side; compare $k_{2,app}$ = 7 ± 2 $M^{-1}$ $s^{-1}$ for d($T_{15}$p(S)T) with 19 ± 3 $M^{-1}$ $s^{-1}$ for d(Tp(S)$T_{15}$) at [cation] = 0.035 M, and $k_{2,app}$ = 1.4 ± 0.3 $M^{-1}$ $s^{-1}$ for d($T_{15}$p(S)T) with 2.4 ± 0.3 $M^{-1}$ $s^{-1}$ for d(Tp(S)$T_{15}$) at [cation] = 0.50 M.

The reactivity profiles obtained in the presence of $Mg^{2+}$ are illustrated in Figure 3b. At high concentrations (0.50 M), the platination rate for d($T_n$p(S)$T_{16-n}$) shows little variation with the position of the phosphorothioate group. An average rate constant of $k_{2,app}$ = 1.2 ± 0.2 $M^{-1}$ $s^{-1}$ can be calculated from the experimental data for these 16-mer oligonucleotides. A decrease of the salt concentration to 35 mM has only a minor influence on the platination rates for the end positions, which were determined to be $k_{2,app}$ = 1.0 ± 0.1 $M^{-1}$ $s^{-1}$ for d($T_{15}$p(S)T) and 1.7 ± 0.2 $M^{-1}$ $s^{-1}$ for d(Tp(S)$T_{15}$). Platination rates of d($T_4$p(S)$T_{12}$), d($T_8$p(S)$T_8$), and d($T_{12}$p(S)$T_4$) are significantly larger however, and the reactivity of these oligomers can be described by a common average of $k_{2,app}$ = 3.6 ± 0.3 $M^{-1}$ $s^{-1}$.

## Discussion

**Concentration Dependence.** The introduction of phosphorothioate groups into normal DNA has proven to be a useful method for the direction of platinum complexes to well-defined binding sites on the charged phosphodiester backbone.[24,25,49,50] The preferential adduct formation with these groups is partially due to kinetic factors,[24] where the softness and negative charge of the S-donor favor formation of the Pt−S bond over adduct formation with the bases, e.g., G−$N_7$. The use of the monoaqua complex **2** as the metalation reagent allows for a reaction mechanism where the rate-determining step for product formation is the direct replacement of the aqua ligand of the complex for the phosphorothioate group. Hydrolysis of the chloride ligand of *cis*-[Pt($NH_3$)($NH_2C_6H_{11}$)Cl($OH_2$)]$^+$ is likely to be negligible within the time frame employed for this study.[14,42,51,52] The linear relation between $k_{obsd}$ and the total concentration of **2**, $C_{Pt}$, or $[Pt(II)]_{tot}$ is in agreement with such a simple reaction mechanism. The absence of an intercept suggests a reaction without significant contribution from the reverse reaction and a rate law according to eq 1 for the reactions of the dinucleotide, d(Tp(S)T)

$$-\mathrm{d}[\mathrm{d}(T_n\mathrm{p(S)}T_m)]/\mathrm{d}t = k_{2,app}[\mathrm{d}(T_n\mathrm{p(S)}T_m)][\mathrm{Pt(II)}]_{tot} \quad (1)$$

($n = m = 1$), and the various oligonucleotides, d($T_n$p(S)$T_m$) ($n$ = 1, 4, 8, 12, 15 and $m = 16 - n$).

**Reactivity Profiles.** The plots in Figure 3 give a more detailed picture of how the reactivity is influenced by the local environment of the p(S)-site within an oligonucleotide of given size with overall charge $|Z| = 15$. In the presence of mono-

charged cations, a preference for platination of the middle position is observed at both 35 mM and 0.50 M cation concentrations; see Figure 3a. In contrast, with $Mg^{2+}$ in the supporting electrolyte, little kinetic discrimination is found between the embedded p(S)-linkages in d($T_n$p(S)$T_{16-n}$), $n = 4$, 8, and 12. At the lower salt concentration employed, 35 mM, the reactivity of these embedded groups is still significantly higher compared with that obtained for the end positions. For higher salt concentrations, however, all phosphorothioate sites exhibit a similar reactivity; see Figure 3b.

The choice of the poly-d(T) fragment as the common backbone for the present study excludes contributions to the reactivity from variations of the base sequence. This is particularly true for the p(S)-linkages in d($T_n$p(S)$T_{16-n}$), $n = 4$, 8, and 12, which are all embedded in an identical surrounding of at least eight bases: d(TTTTp(S)TTTT). In the absence of sequence effects, the present medium-dependent variations in reactivity must therefore be due to molecular properties of the oligomer. It is tempting to conclude that the variations in reactivity simply reflect a varying tendency for local accumulation, or condensation, of cations along these short oligomers, presupposed that the oligomers used exhibit polyanionic properties dominated by end effects.[35−37] This conclusion is supported by recent theoretical calculations on short double-stranded oligomers with an overall charge in the range $8 \leq |Z| \leq 34$.[53] The calculations predict a maximum local concentration of monovalent cations at the middle position of these oligomers. However, after complex formation with an octavalent ligand $L^{8+}$ the local surface concentration of monovalent cations was found to be reduced to a common value for the base pairs in the central part of the studied oligomers. Both of these observations are in good agreement with our experimental findings, provided that the influence of the divalent $Mg^{2+}$ is to reduce the net charge of the internal phosphates in a way similar to that of $L^{8+}$. This function of $Mg^{2+}$ agrees with its well-known higher affinity for polyanions and stronger structural influence compared with monovalent cations with a smaller charge/radius ratio, e.g., $Na^+$.[54,55]

**Influence from the Polymer Environment.** As shown earlier, the rate of adduct formation between **2** and phosphorothioates is accelerated after incorporation of the target phosphorothioate linkage into a polymeric DNA environment.[24,25,28] The present investigation shows that this effect depends not only on the ionic strength of the medium but also on factors such as the net charge of the medium cations and the location of the binding site within the oligonucleotide fragment. The effect is illustrated by the ca. 200- and 13-fold larger rate constants obtained for platination of d($T_8$p(S)$T_8$) compared with those obtained for d(Tp(S)T) at 1.5 mM and 0.2−0.5 M monovalent bulk cation concentration, respectively. It is also noteworthy that the end positions, i.e., d(Tp(S)$T_{15}$) and d($T_{15}$p(S)T), exhibit DNA-promoted reactivity despite the reduced tendency for cation accumulation expected in their vicinity;[35−37] see Figure 2b. For $Mg^{2+}$ in the reaction medium, on the other hand, the absolute rates as well as the rate enhancements are much smaller; see Figure 2. For example, the maximum rate enhancements, still observed for platination of d($T_8$p(S)$T_8$), are reduced to a factor of ca. 70 and 3 at 1.5 mM and 0.20−0.50 M cation concentration, respectively. Finally, platination of the end positions shows DNA-promoted reactivity for the low salt concentrations only, a factor of ca. 16.

**Table 2.** Slopes ($Z_AZ_B$) and Intercepts (log $k_0$) after Analysis of Salt Dependence for the Reactions of d($T_8$p(S)$T_8$) and d(Tp(S)$T_{15}$) with **2** in the Presence of Monovalent and Divalent Cations[a]

| oligonucleotide | predominant cation[b] | $Z_AZ_B$ | log $k_0$ |
|---|---|---|---|
| d($T_8$p(S)$T_8$) | $Na^+$ | $-4.9 \pm 0.5$ | $2.5 \pm 0.2$ |
| | $Mg^{2+}$ | $-3.6 \pm 0.8$ | $1.6 \pm 0.2$ |
| d(Tp(S)$T_{15}$) | $Na^+$ | $-5.0 \pm 0.8$ | $1.8 \pm 0.2$ |
| | $Mg^{2+}$ | $-4.0 \pm 0.2$ | $1.2 \pm 0.1$ |

[a] Analysis based on observation in the interval $0.0015 \leq I \leq 0.3$ M. [b] $[K^+] = 5$ mM for [cation] = 35 mM, and 0.50 mM for all other [cation].

Together these data show that a change from monocharged to doubly charged cations in the supporting electrolyte has a large influence on the observed reactivity, resulting in a reduced kinetic influence from the surrounding oligonucleotide. Two effects can be noticed. First of all, the observed rate constants reveal a reduction of the apparent nucleophilicity of the phosphorothioate group at constant salt concentration and ionic strength for reaction with the cationic complex **2**. Second, a decreased response to a change of the bulk salt concentration is observed.

**Mechanistic Considerations.** Mechanistic interpretation of kinetics data for interactions with groups located on short oligonucleotides is hampered by the lack of detailed information on the solution behavior of such DNA fragments. In particular for reactions with cations such as **2**, the role of the electrostatic interactions with the negatively charged DNA backbone have not been fully elucidated. It is clear from the salt and length dependence[25,28] that the kinetics are influenced by the presence of nonreacting phosphodiester linkages on the target oligomer. There are several ways to analyze and interpret the available data. We will discuss two alternatives below: (i) analysis according to the kinetic salt effect and (ii) assumption of a salt-dependent preassociation of **2** combined with the release of monovalent cations from the oligomer surface. Both approaches support a mechanism in which the cationic complex **2** (net charge $|Z| = 1$) interacts electrostatically with a diffuse negative surface of the DNA ($|Z| > 3$), prior to the rate-determining adduct formation step.

**Kinetic Salt Effect.** The influence from the ionic strength, $I$, on the rate constant for the reaction of **2** with d($T_8$p(S)$T_8$) and d(Tp(S)$T_{15}$) was analyzed by plots of log($k_{2,app}$) vs $I^{1/2}/(1 + I^{1/2})$, according to literature.[56] A summary of the numerical values obtained for the product of ionic charges of the reactants, $Z_AZ_B$, and the logarithm of the rate constants at infinite ionic strength, log $k_0$, is given in Table 2. The negative values determined for $Z_AZ_B$ are expected considering the opposite charges of the reactants. The absolute values ranging from −5 to −3.6 are significantly more negative compared with the value of −1 expected for a direct and exclusive interaction of **2** with a single monoanionic phosphodiester linkage, however. Further, a change of bulk cations from $Na^+$ to $Mg^{2+}$ was found to reduce the magnitude of the charge interactions between **2** and both

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oligomers. Such reduction is suggestive of $Mg^{2+}$ as the better electrostatic screening agent when present in the condensation layer and fits well with its documented both larger binding affinity to DNA and larger charge-to-radius ratio compared with $Na^+$.

A simplistic interpretation of these data includes a mechanism where platination is facilitated by the formation of an outer-sphere complex prior to the rate-determining step: $Pt^+ + DNA \rightleftarrows Pt^+\cdots DNA$. In this reaction, the surrounding phosphodiester linkages are assumed to contribute to an increase of the equilibrium constant for the reaction, $K_{os}$, compared with the similar preassociation constant that can be defined for the reaction with the reference system d(Tp(S)T). The similar products of $Z_AZ_B$ obtained for reactions with the middle and the end positions suggest that the nature of the charge interactions reflects an average electrostatic property of the oligomer, or parts of it, rather than the electrostatic environment in the direct vicinity of the target site. In addition, the dependence of $Z_AZ_B$ on the choice of counterions in the medium ($Na^+$ or $Mg^{2+}$) suggests that this average electrostatic property, or diffuse charge, is a function not only of the DNA molecule itself but also of the type of cations associated with the DNA.

**Salt-Dependent Preassociation Combined with Release of Associated Cations.** The presence of associated cations on the surface of the studied oligonucleotides is expected due to their polyanionic nature. For such polymers, the amount of associated or condensed cations can be regarded as an average inherent molecular property determined by the axial charge density.[15,18] If the formation of the outer-sphere complex, vide supra, is assumed to be driven mainly by the electrostatic interactions created by such charge density, rather than exclusive interaction with a single phosphodiester linkage, association of **2** is expected to be accompanied by release of another cation from the oligomer surface. A modified elementary reaction for this preassociation step is given in eq 2. The use of counterion

$$Pt^+ + DNA\cdots Na^+ \rightleftharpoons Pt^+\cdots DNA + Na^+ \qquad K_{ass} \quad (2)$$

condensation theory (CC) allows for determination of the net charge of the interacting "ligand", in this case the Pt(II) complex, by a plot of $\log(K_{ass})$ vs $\log(c_1)$, where $c_1$ denotes the concentration of supporting monovalent electrolyte.[15] The net charge of the reactive complex can thus be evaluated in the present systems assuming that the variations in $k_{2,app}$ simply reflect the salt dependence of the equilibrium in eq 2, i.e., a variation of the fraction of **2** in the system entering the formally monomolecular reaction pathway leading to rate-determining adduct formation step, eq 3. An analysis of the present data by

$$Pt^+\cdots DNA \rightarrow Pt^+\text{-}DNA \qquad k_2 \quad (3)$$

use of a plot of $\log(k_{2,app})$ vs $\log[Na^+]$ results in an average net charge of the platinum complex of $0.7 \pm 0.2$ for the reactions with $d(T_8p(S)T_8)$ and $d(Tp(S)T_{15})$, close to the expected value of 1.[15] The resulting expressions for the reaction rate and the observed rate constant are given by eqs 4 and 5.

$$-d[DNA]/dt = k_2K_{ass}[Pt^+]_{tot}[DNA\cdots Na^+]/[Na^+] \quad (4)$$

$$k_{obs} = k_2K_{ass}[Pt^+]_{tot}/[Na^+] \quad (5)$$

A plot of the inverse of $k_{2,app}$ as a function of $[Na^+]$ is given in Figure 4a for the reactions with $d(T_8p(S)T_8)$ and $d(Tp(S)T_{15})$ together with a best fit of a linear equation to the data. The larger slope obtained for $d(Tp(S)T_{15})$, $3.4 \pm 0.3$, indicates a smaller value for the product $k_2K_{ass}$ during platination of the end position compared with platination of $d(T_8p(S)T_8)$, with a slope of $0.5 \pm 0.1$. This observation fits well with a model where the central part of the oligomer is the preferred region for nonspecific electrostatic interactions,[35,37,53,57] as already discussed in connection with the reactivity profiles.

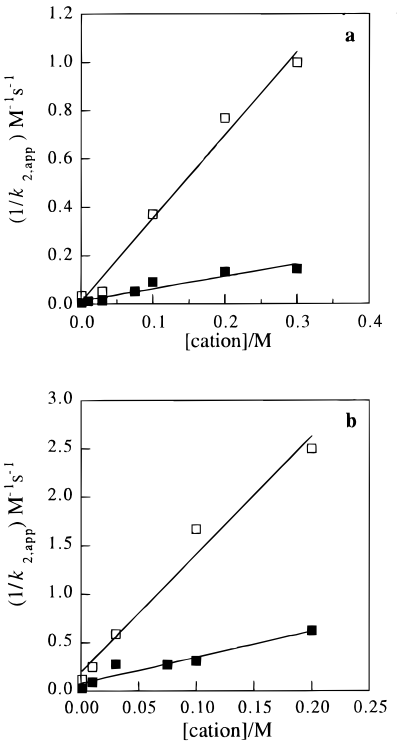


**Figure 4.** Plots of the inverse of the apparent second-order rate constant vs bulk cation concentration for the reaction of **2** with $d(Tp(S)T_{15})$ (□) and $d(T_8p(S)T_8)$ (■), at pH $4.1 \pm 0.1$ and 25.0 °C, $[Pt(II)] = (3.78-27.6) \times 10^{-5}$ M, and $[oligonucleotide] = (4.00-18.0) \times 10^{-6}$ M at (a) variable concentration of $Na^+$ and (b) variable concentration of $Mg^{2+}$.

The data obtained with $Mg^{2+}$ in the supporting electrolyte are also reasonably well described by a linear relationship. Thus, there is experimental support for a reaction mechanism as that outlined in eqs 2 and 3 also in the presence of $Mg^{2+}$. Again, the plot for platination of $d(Tp(S)T_{15})$ exhibits the largest slope, $12 \pm 2$, compared with $2.7 \pm 0.4$ for platination of $d(T_8p(S)T_8)$. The larger values obtained for $Mg^{2+}$ solutions can be rationalized if the contribution from the preassociation step, $k_2K_{ass}$, is smaller in the presence of $Mg^{2+}$ compared with the monovalent cation situation. This assumption seems reasonable considering the better binding affinity of $Mg^{2+}$ to the charged oligomer backbone,[58] which is likely to effectively prevent formation of the productive outer-sphere complex leading to the final $Pt^+$–DNA adduct, eq 3.

## Conclusions

Rate constants for platination of small-size phosphorothioate-containing single-stranded oligonucleotides in aqueous media are strongly dependent on binding site location and on the nature and concentration of the neutral salts dissolved in the medium. Platination is typically favored by (i) a binding site located in the middle of the oligonucleotide, (ii) low salt concentration in the reaction medium, and (iii) a monocharged cation surrounding. In contrast, the dimer d(Tp(S)T) shows little variation in

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reactivity when exposed to similar changes of the reaction medium. The different kinetic behavior of the dimer compared with the oligomers can be accounted for by a reaction model in which preassociation of the platinum complex on the oligomer is required for the formation of the metal adduct. The observed variations in reactivity as a function of the position of the binding site along the DNA fragment can further be interpreted as a varying tendency for cation accumulation along the phosphodiester backbone. Formation of the outer-sphere adduct is likely to be accompanied by release of cations from the oligomer surface. The present findings are relevant for a general understanding of the solution behavior of short DNA fragments, and they are of particular importance for the interpretation of sequence-dependent kinetics for metal binding to DNA model systems. In addition, the results should be of interest for applications both in vitro and in vivo, e.g., for the optimization of synthetic procedures and the use of antisense technologies as a tool for detection and treatment of genetically caused diseases.

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**Supporting Information Available:** Observed pseudo-first-order rate constants as a function of salt concentration, numerical values with errors for the slopes in Figure 4, and representative HPLC traces. This material is available free of charge via the Internet at http://pubs.acs.org.

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