Platinum Binding to d(GpG) Target Sequences and Phosphorothioate Linkages in DNA Occurs More Rapidly with Increasing Oligonucleotide Length

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DNA creates a microenvironment in solution different from that of the bulk. Cations and hydrogen-bond donors are selectively attracted to the negatively charged surface of the polymer,[1] and the hydrophobic core created by the stacked base pairs further provides a compartment for binding intercalators.[2] The DNA microenvironment modulates the rate and mechanism for a variety of chemical reactions. Enhanced reactivity on DNA has been reported for protein diffusion to target sites,[3] electron transfer,[4,5] and substitution reactions.[6-9]

Orientation effects, where the double helix fixes the geometry of the reactants, can be a significant factor in DNA-promoted reactions.[9] Electrostatic interactions are also likely to be of importance, however. In the case of the antitumor drug *cis*-$[Pt(NH_3)_2Cl_2]$ binding to d(GpG) sequences, its preferred target sites on DNA,[10-12] the *cis*-$[Pt(NH_3)_2Cl(OH_2)]^+$ cation has emerged as the key active intermediate.[13] Consequently, the mechanism may involve aggregation of *cis*-$[Pt(NH_3)_2Cl(OH_2)]^+$ cations onto the surface of DNA. Provided that such aggregation is accompanied by a high degree of mobility along the polymer backbone, this effect alone could significantly enhance the rate of platination. Moreover, such facilitated reactivity on a linear polymer could account for the targeting of DNA, rather than RNA, proteins, or nucleotides, by cisplatin in the tumor cell. In the present report we provide quantitative evidence that such a DNA-promoted effect is occurring, through studies of the reactions of platinum(II) amine halides with the N7 atoms of a pair of adjacent guanine bases in the d(GpG) sequence and with phosphorothioate-containing oligonucleotides. The latter are frequently applied as mechanistic probes in DNA and RNA biochemistry[14,15] as well as antisense inhibitors of protein expression.[16] For both cases, experiments were carried out to investigate the effect of DNA length on the rate of platination.

The *cis*-$[Pt(NH_3)(NH_2C_6H_{11})Cl(OH_2)]^+$ cation, a metabolite of the orally administered anticancer drug *cis,trans,cis*-$[Pt(NH_3)(NH_2C_6H_{11})(OC(O)C_3H_7)_2Cl_2]$,[17,18] was used to follow the kinetics of platinum binding.[19] This monocationic aqua complex is likely to associate electrostatically with the anionic phosphate polymer backbone without intercalation.[17] When *cis*-$[Pt(NH_3)(NH_2C_6H_{11})Cl(OH_2)]^+$ was allowed to react with d(GpG) and $d(T_7GGT_7)$, the disappearance of the unplatinated d(GpG)-containing oligonucleotide could be followed by HPLC. This reaction corresponds to the first step in the formation of intrastrand d(GpG) cross-links, namely, monofunctional platinum binding to N7 of guanosine. A summary of pseudo-first-order and apparent second-order rate constants is provided in Table 1.

Reaction of the two diastereomers of d(Tp(S)T) and $d(T_8p(S)T_8)$[20] with *cis*-$[Pt(NH_3)_2Cl(OH_2)]^+$, *cis*-$[Pt(NH_3)(NH_2C_6H_{11})Cl(OH_2)]^+$, or $[Pt(terpy)Cl]^+$ [21] was followed qualitatively by use of ^{31}P NMR spectroscopy. Platination gave rise to 17–22 ppm unfield chemical shifts of the phosphorothioate ^{31}P resonances depending on the platinum complex used (Table S1 and Figure S2, supplementary material). Two signals were observed in both ^{31}P and ^{195}Pt NMR spectra following platination with the monofunctional $[Pt(terpy)Cl]^+$ cation, corresponding to Pt–S bond formation for both diastereomers. A more complicated splitting pattern appeared in the ^{31}P NMR spectrum after platination with the bifunctional complexes *cis*-$[Pt(NH_3)_2Cl(OH)_2]^+$ and *cis*-$[Pt(NH_3)_2(NH_2C_6H_{11})Cl(OH)_2]^+$. This result is in agreement with the more complex distribution of products expected following hydrolysis of the chloride ion.

As shown in Figure 1, the rate constants for platination of both d(Tp(S)T) and $d(T_8p(S)T_8)$ depend linearly on the concentration of added *cis*-$[Pt(NH_3)(NH_2C_6H_{11})Cl(OH_2)]^+$. The apparent second-order rate constants, k_2, determined from the slope of the line, are 0.080 ± 0.016 M^{-1} s^{-1} for platination of d(Tp(S)T) and 3.1 ± 0.4 M^{-1} s^{-1} for platination of $d(T_8p(S)T_8)$, revealing an approximately 40-fold rate increase for platination of the longer oligonucleotide. The influence of DNA duplex formation on the rate of platination of phosphorothioate-containing oligonucleotides was studied at 0 °C.[22] No difference between the reactivity of double- and single-stranded material was observed. Sample kinetic traces are shown in Figure S4 (supplementary material), and kinetic constants are presented in Table 1.

As can be seen from the results in Table 1, the formation of both platinum–d(GpG) and Pt–S phosphorothioate linkages occurs with a rate constant that is 35–40 times larger for the hexadecaoligonucleotides than for the dinucleoside monophosphates. These differences in reactivity are, to our best knowledge, the first reported examples of bimolecular substitution reactions accelerated as a result of weak interactions with the DNA surface. The 40-fold rate increase is of the same order of magnitude as the previously estimated lower limit for the enhanced binding of ethidium to *cis*-$[Pt(NH_3)_2Cl(OH_2)]^+$ covalently attached to DNA.[7] Significantly, the enhancement factor is similar to that reported as the upper limit for diffusion-controlled protein–DNA

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(19) The reactions were studied at 25.0 °C in aqueous solution, pH 6.50, under pseudo-first-order conditions with [Pt(II)] in excess. Aliquots of the reaction mixture were removed, diluted with buffer, and freeze-quenched. The thawed aliquots were analyzed by reverse phase HPLC. Pseudo-first-order rate constants for reactant decay and product formation were obtained by fitting the time dependence of HPLC peak areas to a single exponential function (Figure S1). Control experiments with $d(T_{16})$ and *cis*-$[Pt(NH_3)_2(NH_2C_6H_{11})Cl(OH_2)]^+$ as reactants were also performed. In the absence of the specific binding sites, phosphorothioate or d(GpG), no change was observed in the area or retention time of peaks corresponding to the reactants.
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Table 1. Observed Pseudo-First-Order Rate Constants and Corresponding Second-Order Rate Constants for Platination of Guanine- and Phosphorothioate-Containing Oligonucleotides with *cis*-$[Pt(NH_3)(NH_2C_6H_{11})Cl(OH_2)]^+$ [a]

oligonucleotide	[Pt(II)]/M	k_{obs}/s^{-1}	$(k_{obs}/[Pt(II)])/M^{-1}\ s^{-1}$	relative reactivity	$k_2/M^{-1}\ s^{-1}$
d(GpG)	7.93×10^{-3}	$(2.1 \pm 0.3) \times 10^{-4}$	0.026 ± 0.004	1.0	
d(Tp(S)T)	7.95×10^{-3}	$(7.9 \pm 0.6) \times 10^{-4}$	0.099 ± 0.008	3.8	0.080 ± 0.016 [b]
$d(T_7GGT_7)$	3.93×10^{-4}	$(3.57 \pm 0.13) \times 10^{-4}$	0.91 ± 0.04	35	
$d(T_8p(S)T_8)$	3.93×10^{-4}	$(9.9 \pm 1.0) \times 10^{-4}$	2.5 ± 0.3	96	3.1 ± 0.4 [b]

[a] pH 6.50, 25 °C. [b] Determined from the concentration dependence of k_{obs} according to Figure 1. Experimental conditions and complete data are given in Table S2.

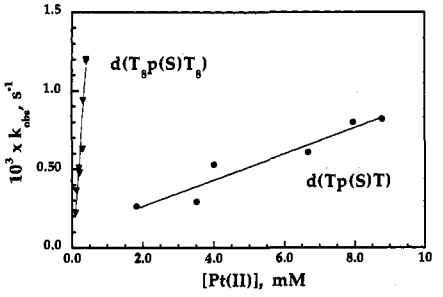

Figure 1. Observed pseudo-first-order rate constants as a function of total concentration of platinum for reaction of (●) d(Tp(S)T) and (▼) and $d(T_8p(S)T_8)$ with *cis*-$[Pt(NH_3)_2(NH_2C_6H_{11})Cl(OH_2)]^+$. Full data are given in Table S2.

interactions.[23] The fact that similar rate enhancements occur for platinum binding to both d(GpG) and phosphorothioate target nucleophiles strongly favors a rate acceleration mechanism independent of the specific DNA adducts. A simple rationalization based on net charge effect alone can be excluded by the similar reactivity observed for reactions with single- and double-stranded DNA. Moreover, we can also eliminate, as the sole explanation for the rate increase, the possibility that the extended oligonucleotide preorganizes the target site compared to a dinucleoside monophosphate. In a preliminary experiment, the reactivity of $d(T_4p(S)T_4)$ with *cis*-$[Pt(NH_3)(NH_2C_6H_{11})Cl(OH_2)]^+$ was measured to be intermediate between those of d(Tp(S)T) and $d(T_8p(S)T_8)$.[24] Instead, we favor an explanation that invokes a decrease in the effective reaction volume arising from preassociation of the cationic platinum complex with the negatively charged DNA surface. The increased local concentration of the Pt(II) complex in the vicinity of the biopolymer, combined with a relatively high degree of mobility of the complex along the polymer backbone, lead to the observed rate enhancement.[25]

Little quantitative information is available about the reactivity of phosphorothioates as nucleophiles toward metal ions. Their early use as specific sites for metalation of DNA and RNA,[21,26] and their documented ability to create specific cross-links in the presence of platinum(II) complexes within duplex and triplex DNA,[27,28] and between transcription factors and their recognition sequences,[29] revealed a preference for selective binding of soft metal ions to the phosphorothioate sulfur atom rather than to a nucleobase. The present results demonstrate this selectivity to be due in part to kinetic factors, with an approximately 3-fold higher rate constant for platination of the phosphorothioate site compared to the d(GpG) site, irrespective of the oligonucleotide length (Table 1).

In conclusion, the experiments reported here highlight the importance of weak interactions between metal ions and DNA, especially at the diffuse interface between the bulk solution and the biopolymer surface, in facilitating covalent binding that is usually a requirement for an effective probe or drug. Knowledge of this phenomenon and its effect on reaction mechanisms should be valuable for optimizing drug–DNA interactions, predicting and modulating toxicity levels, and designing new metal-based therapies that involve coordination to DNA in vivo.

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Supplementary Material Available: Figure S1 comparing experimental and calculated kinetic traces for platination of di- and hexadecaoligonucleotides, Table S1 and Figure S2 reporting ^{31}P and ^{195}Pt NMR data, Table S2 listing experimental conditions and rate constants for platination of $d(T_np(S)T_n)$, Figure S3 showing the melting curve for $d(A_{16})\cdot d(T_8p(S)T_8)$, and Figure S4 displaying experimental and calculated kinetic traces for platination of $d(T_8p(S)T_8)$ and $d(A_{16})\cdot d(T_8p(S)T_8)$ (11 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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