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Reactions of Pd(dien)Cl^+ with thione-containing nucleosides, nucleotides and oligonucleotides: increase of both enthalpy and entropy of activation in the DNA-environment

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Dedicated to Professor Stephen J. Lippard on the occasion of his 60th birthday.

Abstract

Reactions of chloro(diethylenetriamine)palladium(II) with 4-thio-2′-deoxyuridine, 4-thiouridine-5′-monophosphate, d($T_8^{s4}UT_8$), 6-thio-2′-deoxyinosine, 6-mercapto-purine riboside-5′-monophosphate and d($T_8^{s6}IT_8$) have been investigated using diode-array and conventional stopped-flow spectroscopy in acidic aqueous solution as a function of excess concentration of Pd(II) and temperature. The observed rate constants and activation parameters indicated a reaction mechanism where the labile chloro ligand is directly replaced by the thione unit in the coordination sphere of the Pd(II) complex. Incorporation of both thionucleosides into single-stranded oligonucleotides was found to increase the rate of adduct formation by a factor of ca. 2–3, compared to reactions of the corresponding mononucleotides. Compared to the monomer reactions, adduct formation in DNA-environment results in an increase of both the $\Delta H^{\neq}$- and $\Delta S^{\neq}$-values. The observed DNA-promoted reactivity is thus a consequence of the reduced contribution to the activation energy from the $\Delta S^{\neq}$-term.

Keywords: Kinetics and mechanism; Palladium complexes; Nucleoside complexes; Nucleotide complexes

1. Introduction

Interactions of freely diffusing transition metal complexes with functional groups located on oligodeoxyribonucleotides exhibit reaction characteristics different from that of simple model systems. Reactions of rather inert cationic metal complexes, e.g. Pt(II) or Ru(IV), have been found to be significantly accelerated in the DNA environment, even when the sizes of the target oligomers are limited [1–8]. A salt dependence has been observed for interactions of both rapidly and slowly reacting metal complexes with functional groups located on both the phosphodiester backbone and on the bases [2,3,8–11]. Based on these observations, a general influence from an electrostatically driven pre-association of the charged metal complex onto the surface of the polyanionic oligonucleotide has been suggested as an important step in the reaction mechanism for metal binding to DNA. The presence of such a pre-equilibrium may explain the targeting of extended DNA in the cell by cisplatin rather than adduct formation with the mononucleotide pool, short RNA fragments or other cell components [12–15]. The rather modest reactivity of the platinum center allows such diffusion controlled association to take place without competition from the rate determining adduct formation process. In order to provide an efficient reaction path for cationic reagents in general, the mobility of the associated

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cations has to remain close to diffusion control, for example by rapid exchange between the condensation layer and fully hydrated cations in the second hydration sphere of the DNA [16–21]. In addition, the influence from steric interactions around the target site should not hamper the close interaction of the metal center with the donor site on the DNA.

The present study of the complex $Pd(dien)Cl^+$ as a metallation reagent has been undertaken to address the question of general validity of the pre-association step. The palladium complex has a reactivity that exceeds that of platinum(II) complexes by about five orders of magnitude [22,23], which imposes more demanding conditions for both the rate of pre-association and the subsequent mobility in the DNA-environment in order for this reaction path to contribute to the adduct formation process. Furthermore, the use of the chelating dien-ligand adds a structural constraint to formation of both the transition state and the products, compared with the related investigations of Au(III) reactions carrying ammine or cyanide ligands [24,25]. Studies of the interactions between DNA and sterically hindered Pt(II) complexes have shown that such subtle changes in the coordination environment could have a large impact on the distribution of final adducts [26,27], but the magnitude of this influence on the adduct formation rate remains to be elucidated.

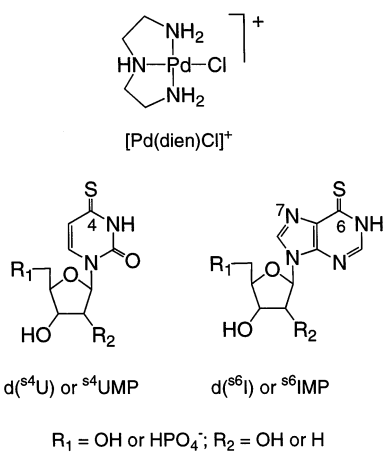
The nucleosides, nucleotides and oligonucleotides used in the present study contain the common thiocarbonyl group, bound to the C4-position on a pyrimidine base or C6-position on a purine base, e.g. 4-thio-2′-deoxyuridine and 6-thio-2′-deoxyinosine, see Chart 1. In both cases the sulfur atom provides a soft and kinetically preferred target site for the palladium(II) center compared with other available donor atoms. 4-Thiouridine is a natural constituent of *Eschericia coli* tRNA and 6-mercaptopurine is used for clinical treatment of leukemia [28–31]. Both types of sulfur donors have been shown to interact specifically with metal centers of Tl(III), Hg(II), Au(III) and Pt(II) [24,25,32–34]. The kinetic influence from a single neighboring charged phosphate group was investigated by comparison of the reactivity of the non-charged nucleosides d(^{s4}U) and d(^{s6}I) with that of the nucleotides ^{s4}UMP and ^{s6}IMP. The influence from a polyanionic environment was examined using a single-stranded DNA containing a single ^{s4}U or ^{s6}I target site; d($T_8^{s4}UT_8$) or d($T_8^{s6}IT_8$). The adduct formation rate of the thione moieties on these oligonucleotides was found to be accelerated in comparison to reactions with the monomers by a factor of 2–4. The relative increases in reactivity are similar to those recently reported for reactions of Au(III) complexes. Support is thus given for a common contribution to the DNA-promoted effect from processes more rapid than the adduct formation step.

2. Experimental

2.1. Chemicals and solutions

Buffer solutions were prepared from aqueous stock solutions of potassium hydrogen phthalate $KHC_8H_4O_4$ (Baker, AR) or NaH_2PO_4 (Sigma, RG), $NaClO_4$ (Merck, pa), or NaOH (Eka, pa) by slight modification of literature procedures [35] to maintain a constant ionic strength of 0.20 M in all experiments. Buffers with $pH \leq 5.5$ were made from the stock solution of $KHC_8H_4O_4$ and buffers with $pH \geq 6.0$ from the stock solution of NaH_2PO_4, in both cases by addition of NaOH. A constant chloride ion concentration of 50 mM was achieved by addition of NaCl (Merck, pa) to the standard buffer solutions followed by addition of $NaClO_4$ to adjust the ionic strength. Water was doubly distilled from quartz.

The salt $[Pd(dien)Cl]ClO_4$ (dien = $NH_2C_2H_4NHC_2H_4NH_2$) was prepared from $PdCl_2$ (Johnson Matthey) according to literature procedures (Chart 1) [36]. Calc. for $[Pd(dien)Cl]ClO_4$: C, 13.95; H, 3.80; N, 12.20.



(Chart 1)

Found: C, 13.9; H, 3.9; N, 12.1%. Stock solutions were prepared directly before use by dissolving a weighed amount of $[Pd(dien)Cl]ClO_4$ in buffer containing 50 mM chloride to suppress the formation of phthalate, phosphate and aqua complexes. The concentration of such complexes in the solutions was negligibly small for the experimental conditions used, as estimated using available stability constants for oxygen-donor complexes [37,38].

4-Thio-2′-deoxyuridine, d(^{s4}U), 6-thio-2′-deoxyinosine, d(^{s6}I), d($T_8^{s6}IT_8$) and d($T_8^{s4}UT_8$) were prepared as described before (Chart 1) [39–41]. Stock solutions of d(^{s4}U), 4-thiouridine-5′-monophosphate (^{s4}UMP) (Sigma), d(^{s6}I), 6-mercaptopurine riboside-5′-monophosphate (^{s6}IMP) (Sigma), d($T_8^{s6}IT_8$) and d($T_8^{s4}UT_8$), were prepared in buffered solution. Concentrations were determined spectrophotometrically at 322 nm for d(^{s6}I), ^{s6}IMP and d($T_8^{s6}IT_8$) or at 332 nm for

d(^{s4}U), ^{s4}UMP and d($T_8^{s4}UT_8$), corresponding to the absorbance maximum of the thione moiety [42–44]. Stock solutions were kept refrigerated in the dark.

2.2. Kinetics

All reactions were monitored under pseudo-first-order conditions, with at least a ten-fold excess of metal complex. The reactions were started by the mixing of equal volumes of a solution of the Pd(II) complex with a solution of the nucleoside, nucleotide or oligonucleotide directly in the stopped-flow instrument. The reaction of the thione-moiety with the metal complex was monitored as a decrease of absorbance at their respective absorbance maximum at either 322 or 332 nm [42–44]. Observed rate constants were calculated by a fit of a single exponential function to the kinetic traces. Reported pseudo-first-order rate constants, k_{obsd}, are mean values of at least five independent kinetic runs. Second-order rate constants, k_{app}, were obtained by a fit of a straight line to the plot of pseudo-first-order rate constants versus total concentration of Pd(II) complex, C_{Pd}, using a standard least-squares minimizing routine. Enthalpies and entropies of activation, $\Delta H^{\neq}$ and $\Delta S^{\neq}$, were obtained by a fit of the natural logarithm of the second-order rate constant, $\ln(k/T)$ versus $1/T$ to the Eyring equation.

2.3. Apparatus

Spectra were recorded using a Milton Roy 3000 diode-array spectrophotometer and thermostated 1.00 cm Quartz Suprasil cells. Time-resolved spectra and kinetics for the reaction with the thione containing nucleosides and nucleotides were recorded at ambient pressure (5–50°C) using an Applied Photophysics Biosequential SX-17MV, stopped-flow ASVD spectrofluorimeter. Spectra and kinetics for the reaction with oligonucleotides were run with a Hi-Tech SF-61 DX2 double mixing diode-array stopped-flow system. Kinetic runs were evaluated by on-line least-squares minimizing programs; SX-17MV Applied Photophysics Software [45] or KinetAsyst V1.5a for Microsoft Windows [46].

3. Results and discussion

3.1. Reactions of d(^{s4}U), ^{s4}UMP, d(^{s6}I) and ^{s6}IMP with Pd(dien)Cl$^+$

3.1.1. Kinetics at constant pH

Replacement of the exocyclic C4 carbonyl in uridine or C6 carbonyl in inosine by sulfur introduces an absorbance peak with a maximum at 332 (^{s4}U) and 322 nm (^{s6}I) well separated from the one typical for the common DNA bases at ca. 260 nm [42–44,47]. Addition of the complex [Pd(dien)Cl]$^+$ to solutions containing these thiones results in the disappearance of the absorbance maximum associated with the thione, and a concomitant increase in the absorbance in the region around 250 nm and above 340 nm. The kinetics was found to depend on the concentration of the added excess Pd(II) complex, and the kinetic traces were well described by single exponential functions (see Fig. 1). The obtained pseudo-first-order rate constants were directly proportional to the total concentration of the metal complex in the pH range 4.8–7.0. Typical plots for the reactions with the uncharged d(^{s4}U) and d(^{s6}I) monomers are shown in Fig. 2 for the reactions at pH 4.8 (complete primary data in Section 4, Table S1). The absence of an intercept allows for a simple interpretation of these reactions as a direct ligand exchange of chloride for thione, Eq. (1), where L = d(^{s4}U), ^{s4}UMP, d(^{s6}I) or ^{s6}IMP. The corresponding rate law and expres-

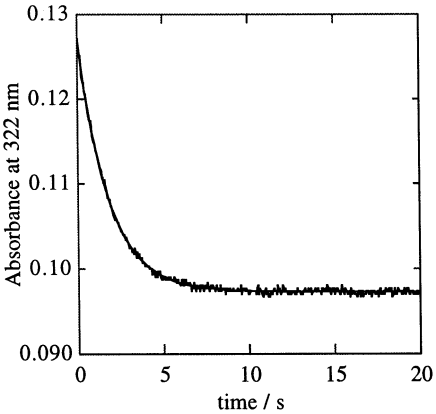


Fig. 1. Kinetic trace and fit to a single exponential function for the reaction of Pd(dien)Cl$^+$ with d(^{s6}I) at 25°C and pH 4.8; $C_{Pd} = 1.0 \times 10^{-4}$ M, [Cl$^-$] = 0.050 M, [d(^{s6}I)] = 5×10^{-6} M, $I = 0.20$ M.

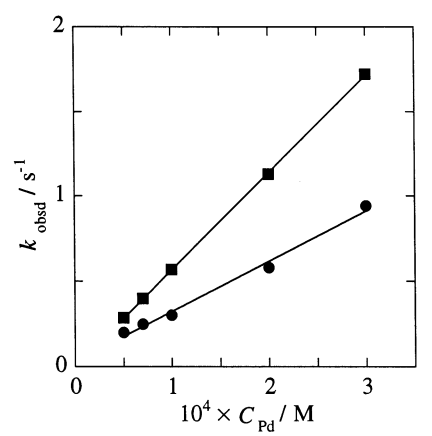


Fig. 2. Observed pseudo-first-order rate constants for the reaction of [Pd(dien)Cl]$^+$ with (●) d(^{s4}U) or (■) d(^{s6}I) as a function of excess Pd(II) concentration, C_{Pd}, at 25.0°C and pH 4.8; [d(^{s4}U], [d(^{s6}I] = 5.0×10^{-6} M, [Cl$^-$] = 0.050 M, $I = 0.20$ M.

Table 1
Rate constants and activation parameters at 25°C for reactions of square-planar complexes with selected sulfur-containing DNA model systems

Reaction	k_{app} (M^{-1} s^{-1})	$\Delta H^{\neq}$ (kJ mol^{-1})	$\Delta S^{\neq}$ (J mol^{-1} K^{-1})
$[Pd(dien)Cl]^+ + d(^{s4}U)$ [a]	$(2.9 \pm 0.2) \times 10^3$	24 ± 1	-99 ± 10
$[Pd(dien)Cl]^+ + {}^{s4}UMP$ [a]	$(3.5 \pm 0.2) \times 10^3$	32 ± 1	-69 ± 10
$[Pd(dien)Cl]^+ + d(T_8^{s4}UT_8)$ [a]	$(8.0 \pm 0.1) \times 10^3$	51 ± 8	3 ± 10
$[Pd(dien)Cl]^+ + d(^{s6}I)$ [a]	$(5.72 \pm 0.04) \times 10^3$	35 ± 2	-55 ± 5
$[Pd(dien)Cl]^+ + {}^{s6}IMP$ [a]	$(8.7 \pm 0.2) \times 10^3$	31 ± 2	-65 ± 5
$[Pd(dien)Cl]^+ + d(T_8^{s6}IT_8)$ [a]	$(2.4 \pm 0.1) \times 10^4$	41 ± 6	-21 ± 9
trans-$[Au(NH_3)_2Cl_2]^+ + d(^{s4}U)$ [b]	$(1.6 \pm 0.1) \times 10^4$	21.7 ± 1.1	-90 ± 4
trans-$[Au(NH_3)_2Cl_2]^+ + {}^{s4}UMP$ [b]	$(1.6 \pm 0.2) \times 10^4$	22 ± 3	-92 ± 9
trans-$[Au(NH_3)_2Cl_2]^+ + d(T_8^{s4}UT_8)$ [c]	$(3.8 \pm 0.2) \times 10^4$	36 ± 3	-30 ± 10
trans-$[Au(NH_3)_2Cl_2]^+ + d(^{s6}I)$ [c]	$(5.5 \pm 0.9) \times 10^4$	34 ± 3	-38 ± 10
trans-$[Au(NH_3)_2Cl_2]^+ + d(T_8^{s6}IT_8)$ [c]	$(3.0 \pm 0.5) \times 10^5$	34 ± 2	-31 ± 6
cis-$[Pt(NH_3)(NH_2R)Cl(OH_2)]^+ + d(Tp(S)T)$ [d]	0.5 ± 0.3 [e]		
cis-$[Pt(NH_3)(NH_2R)Cl(OH_2)]^+ + d(T_8p(S)T_8)$ [d]	11 ± 1		

[a] This work, $I = 0.20$ M, pH 4.8.
[b] From Ref. [24], $I = 0.11$ M, pH 4.0.
[c] From Ref. [25], $I = 0.11$ M, pH 4.0.
[d] From Ref. [8] , $R = C_6H_{11}$, $I = 0.15$ M, pH 4.2.
[e] Data refer to the average rate constant in the interval $0.1 \leq I \leq 0.5$ M.

sion for the observed rate constant k_{obsd} are given in Eqs. (2) and (3), where $k_{app} = k_1$.

$$[Pd(dien)Cl]^+ + L \xrightarrow{k_1} [Pd(dien)L]^{+/2+} + Cl^- \quad (1)$$

$$d[L]/dt = -k_{app}[Pd(dien)Cl^+][L] \quad (2)$$

$$k_{obsd} = k_{app}[Pd(dien)Cl^+] \quad (3)$$

Obviously, the nucleophilicity of these sulfur donors is large enough to suppress the kinetic influence from the solvent path, in contrast to adduct formation reactions with mononucleosides and -nucleotides of the nitrogen donors in the common DNA bases [48–51].

A summary of the obtained second-order rate constants, k_{app}, at 25°C is given in Table 1. Similar rate constants are obtained for the four studied monomers with 3×10^3 M^{-1} $s^{-1} \leq k_{app} \leq 6 \times 10^3$ M^{-1} s^{-1}. A slight preference is found for adduct formation with the thioinosine derivatives $d(^{s6}I)$ and ^{s6}IMP. Adduct formation is slightly but significantly favored for the mononucleotides compared to the corresponding mononucleosides at pH 4.8. This trend is more pronounced as the pH is increased, see Table 2 and discussion below.

A series of second-order rate constants according to $d(^{s4}U) \leq {}^{s4}UMP < d(^{s6}I) \leq {}^{s6}IMP$ can thus be established in the pH-range studied. A similar kinetic preference for adduct formation with the ^{s6}I-moiety was recently reported for reactions of *trans*-$[Au(NH_3)_2Cl_2]^+$ (compare data in Table 1) [25]. However, the rate constants for the complex $Pd(dien)Cl^+$ are about one order of magnitude smaller than those obtained for the gold(III) complex. A somewhat less pronounced discrimination between the two thiones is also observed for the palladium complex. This is expected, since the Pd(II) center has a less pronounced sensitivity towards entering ligands than Au(III) complexes [52]. Despite minor individual variations in observed reactivity, the comparison shows that both metal complexes exhibit a rather similar sensitivity towards the nucleophilic properties of ^{s4}U and ^{s6}I, despite their different coordination environments.

3.1.2. pH dependence

For all monomers, an increase in pH results in an accompanying increase in the second-order rate constants (see Table 2). The detailed response to a pH change in the interval range 4.8–7.0 is clearly different, however. The overall increase in reactivity for the ^{s4}U-moiety is a factor of ca. 2.2–2.7, whereas a factor of ca. 4.2–4.5 is observed for the ^{s6}I-moiety. In both cases, the larger rate increase is observed for the mononucleotide, which suggests that electrostatic interactions with the phosphate group may facilitate the adduct formation process as the pH approaches the pK_a for secondary ionization [47]. A similar pH dependence has been reported for reactions of $Pd(dien)OH_2^{2+}$ with

Table 2
Second-order rate constants for the reaction of $[Pd(dien)Cl]^+$ with thione containing nucleosides and nucleotides as a function of pH

pH	$10^{-3} \times k_{app}$ (M^{-1} s^{-1})			
	$d(^{s4}U)$	^{s4}UMP	$d(^{s6}I)$	^{s6}IMP
4.8 [a]	2.9 ± 0.2	3.7 ± 0.2	5.7 ± 0.1	8.7 ± 0.2
5.6 [a]	3.3 ± 0.2	7.3 ± 0.1	14.2 ± 0.4	24 ± 1
6.0 [b]	3.9 ± 0.1	8.1 ± 0.6	16.2 ± 0.7	25 ± 1
7.0 [b]	6.8 ± 0.2	9.7 ± 0.1	23.9 ± 0.7	37 ± 1

[a] 0.10 M $KHC_8H_4O_4$.
[b] 0.10 M NaH_2PO_4.

cytosine and cytosine monophosphate. In these studies however, the uncharged nucleoside was found to react faster than CMP at pH 7 as a result of competitive phosphate complexation [37]. With Pd(dien)Cl^+ as the reactant, such side reactions seem unlikely, since the phosphate group is a poor nucleophile compared with both free chloride ion (50 mM) and the thiones. The larger increase in reactivity observed for the ^{s6}I-containing monomers suggests that additional interactions could influence the reactivity, e.g. H-bonds between the chelated dien-ligand and the purine N7 atom. However, further studies are needed to deduce these mechanistic details.

3.1.3. Activation parameters

The temperature dependence of the reactions with the thione-containing mononucleosides and mononucleotides was studied at pH 4.8 ranging from 5 or 10 to 45°C (Table S1 and Fig. S1, Section 4). The activation enthalpies and activation entropies fall within the range of $\Delta H^{\neq} = 30 \pm 5$ kJ mol^{-1} and $\Delta S^{\neq}$ -70 ± 25 J K^{-1} mol^{-1}, c.f. Table 1, and are similar to those obtained for reactions of palladium(II) complexes with other sulfur-donor ligands [53,54]. The negative activation entropies suggest that the reactions studied occur according to the commonly observed associatively activated interchange mechanism, I_a [22]. The data in Table 1 further shows that the reactivity sequence obtained at 25°C, d(^{s4}U) ≤ ^{s4}UMP < d(^{s6}I) ≤ ^{s6}IMP, is best described as entropy controlled. For example, the slowest reacting monomer d(^{s4}U) has the smallest $\Delta H^{\neq}$-value, 24 kJ mol^{-1}, but the most negative $\Delta S^{\neq}$-value, -99 J K^{-1} mol^{-1}. For the more rapidly reacting ^{s6}I-containing monomers the opposite is observed: the unfavorable $\Delta H^{\neq}$-values are compensated by less negative $\Delta S^{\neq}$-values of -60 J K^{-1} mol^{-1}.

Despite the similar kinetic behavior of the two thiones, some more subtle differences can be observed primarily for the $\Delta S^{\neq}$-values. For the uncharged thiones, the activation entropies should primarily reflect the reduction in entropy resulting from incorporation of the thione-unit into the inner coordination sphere of the metal complex, with minimal contributions from solvational changes. For the mononucleotides on the other hand, adduct formation is formally associated with charge neutralization. Provided that the distance between the phosphate group and the thione unit is small enough to change the hydration of the reactants in the activation process, an increase in entropy is expected due to loss of hydration. For the ^{s4}U-moiety, such an increase is indeed observed after introduction of the phosphate group, compare $\Delta S^{\neq} = -99 \pm 10$ for d(^{s4}U) with -69 ± 10 J K^{-1} mol^{-1} for ^{s4}UMP. In contrast, for reaction with the ^{s6}I-moiety, the activation entropy is rather insensitive to the presence or absence of the phosphate group with an average $\Delta S^{\neq}$ of -60 ± 10 J K^{-1} mol^{-1}. Factors that might contribute to these differences include the already mentioned variations in H-bonding pattern in the vicinity of the thione. For example, hydrogen bond formation with dI–N7 might influence the reaction with the ^{s6}I-moiety, whereas interactions with the 5′-phosphate group seem to be important only for reaction with the ^{s4}U.

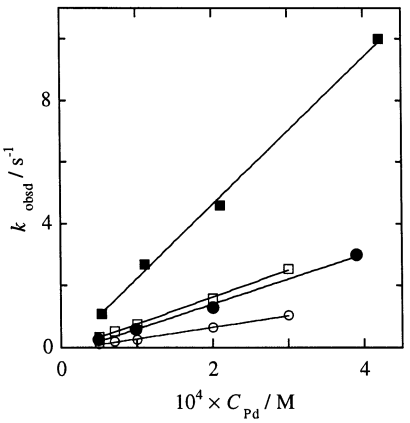


Fig. 3. Observed pseudo-first-order rate constants for the reaction of [Pd(dien)Cl]$^+$ with (○) ^{s4}UMP, (●) d($T_8^{s4}UT_8$), (□) ^{s6}IMP or (■) d($T_8^{s6}IT_8$) as a function of total excess Pd(II) concentration, C_{Pd}, at 25.0°C and pH 4.8; [thione] = (1.8–5.0) × 10^{-6} M, [Cl^-] = 0.050 M, I = 0.20 M.

3.2. Reactions of d($T_8^{s4}UT_8$) and d($T_8^{s6}IT_8$) with Pd(dien)Cl^+

3.2.1. Kinetics at pH 4.8

The reactions of the two oligomers d($T_8^{s4}U_8$) and d($T_8^{s6}IT_8$) with Pd(dien)Cl^+ were studied at pH 4.8 as a function of excess total concentration of palladium(II) and temperature (see Table 1) (experimental data in Section 4, Tables S2 and S3). This pH allows for use of both nucleosides and nucleotides as model systems mimicking the local surrounding of the target site in the oligomers, since the kinetic contribution from secondary ionization of the phosphate groups is minimized [47]. As for the monomers, an exponential decrease of the absorbance at 322 and 332 nm is observed after addition of excess metal complex to a solution containing these oligonucleotides. A plot of the pseudo-first-order rate constants as a function of C_{Pd} for oligomers and the corresponding mononucleotides is shown in Fig. 3. The direct proportionality between k_{obsd} and C_{Pd} suggests that adduct formation in the oligonucleotide environment occurs as a direct displacement of the labile chloride ligand according to Eq. (1), with a rate law of the type given in Eq. (2). Furthermore, incorporation of the thiones into the oligonucleotide fragments increases the apparent reactivity of the thione by a factor of about three for both ^{s4}U and ^{s6}I as targets, compared to the reactions with the monoanionic nucle-

otides ^{s4}UMP and ^{s6}IMP (c.f. Table 1). Apparently, kinetically unfavorable factors related to the size and more demanding stereochemistry of the polyanion are counteracted by other contributions.

DNA-promoted kinetics have been previously observed for reactions involving covalent modification of both specific and non-specific binding sites on various-sized DNA [1–6,8–10,55–58]. For binary systems with well-defined binding sites, most data involve slowly reacting platinum(II) centers [1–6,8]. The investigations include reactions with donor atoms located on the bases as well as on the phosphodiester backbone. Systematic studies of the salt dependence have also been performed, revealing a pronounced influence from the concentration of bulk electrolytes on the rate of adduct formation. A decrease in the rate of adduct formation with increasing concentration of inert bulk electrolytes has been observed for reactions with charged and non-charged target sites located on oligomers of a similar size compared to those in the present study [2,3,8].

For specific interactions of more rapidly reacting metal complexes, the documentation so far seems to be limited to our previous study of the kinetics for the reaction of *trans*-$[Au(NH_3)_2Cl_2]^+$ with the oligomers $d(T_8^{s4}UT_8)$ and $d(T_8^{s6}IT_8)$ [24]. A summary of second-order rate constants for reactions with Pt(II), Pd(II) and Au(III)-centers at comparable salt concentrations is included in Table 1. These data show that the two labile metal complexes $[Pd(dien)Cl]^+$ and *trans*-$[Au(NH_3)_2Cl_2]^+$ exhibit similar rate enhancements (ca. 2–4) for adduct formation with the thiones in the DNA environment. There is a tendency for a slightly larger rate enhancement for reactions with the gold(III) complex, which might be due to the lower salt concentration used in that study, $I = 0.11$ M.

The similar rate increases observed support a reaction mechanism with a common contribution in the oligomer environment. The absence of a correlation between the magnitude of k_{obsd} for the Au(III) and Pd(II) reactions and the observed rate enhancements suggests a contribution from a reaction step with a half life at least one order of magnitude shorter than those observed for the more rapidly reacting gold(III) center, i.e. $t_{1/2} \leq 10$ ms. Plausible alternatives for reactions taking place on this rapid time scale include nearly diffusion controlled processes, such as metal induced reorganization of the oligomer geometry and exchange of cations between the condensation layer and the diffuse Debye–Hückel cloud [16,17,21]. In the light of recent experiments, it seems likely that reorganization and structural changes of the oligomer geometry might contribute to the observed reactivity in the DNA-environment (c.f. discussion of activation parameters below) [59,60]. The similar rate enhancements obtained for the Pd(II) and Au(III) (d^8, square-planar) centers could thus reflect the presence of a mechanism operating only via a direct interaction with the target site, provided that the two metal centers induce similar changes of the molecular geometry around the target site during the activation process. An alternative mechanism consistent with the data proceeds via the already discussed directed diffusion path, by reversible exchange of the associated cationic metal complex with a fully hydrated bulk cation in the close proximity of the DNA [1,2,21].

The mechanistic interpretation of the available kinetics data for adduct formation on short size single-stranded DNA is hampered by factors such as: (i) the lack of predictions concerning the extent of counterion condensation and (ii) the absence of information concerning the microscopic distribution of condensed cations around the DNA [61]. A comparison of the reaction characteristics for the present study with those reported for reactions of *cis*-$[Pt(NH_3)(NH_2C_6H_{11})Cl(OH_2)]^+$ with $d(T_8p(S)T_8)$ indicates that a random distribution of cations along these short oligonucleotides is unlikely (see Table 1). If this was the case, similar-sized DNA should exhibit similar rate enhancements, regardless of the location of the binding site and the inherent reactivity of the metal complex. However, the rate enhancements obtained for adduct formation with the charged phosphodiester backbone are up to one order of magnitude larger compared with the rapid reactions of thione-containing oligonucleotides under similar bulk electrolyte conditions. There are at least two possible explanations for these observations. For a mechanism involving pre-association and directed diffusion along the oligomer, the use of the slowly reacting Pt(II) allows for a greater proportion of the metal complex to operate via the directed reaction pathway. It is also possible that the location of the binding site, as a part of the charged phosphodiester backbone in the reaction with the Pt(II) complex, might contribute in an optimal way to facilitate adduct formation via the direct reaction. Regardless of the interpretation it seems obvious that there is an influence on the magnitude of the rate enhancements by the local environment of the binding site, which favors reactions with donor atoms as part of the charged phosphodiester backbone. A simple mechanism based on these observations is outlined in Eqs. (4) and (5). The influence from the local environment is accounted for in terms of a reversible rapid pre-equilibrium between free and associated Pd(dien)Cl^+ in Eq. (4), followed by rate determining formation of the metal adduct (Eq. (5)).

$$Pd(dien)Cl^+ + d(T_8p(S)T_8) \rightleftharpoons Pd(dien)Cl^+\cdots d(T_8p(S)T_8) \quad K_{ass} \tag{4}$$

$$Pd(dien)Cl^+\cdots d(T_8p(S)T_8) \rightarrow Pd(dien)^{2+}\text{–}d(T_8p(S)T_8) + Cl^- \quad k_2 \tag{5}$$

The rate law and observed rate constants are then given by Eqs. (6) and (7).

$$d[Pd(dien)^{2+}]-d(T_8p(S)T_8)]/dt = k_2K_{ass}[Pd(dien)Cl^+][d(T_8p(S)T_8)] \quad (6)$$

$$k_{obsd} = k_2K_{ass}[Pd(dien)Cl^+] \quad (7)$$

3.2.2. Activation parameters

The activation parameters for the reactions of Pd(dien)Cl^+ with the two oligonucleotides d(T_8XT_8), X = ^{s4}U and ^{s6}I, are included in Table 1. The values of both $\Delta H^{\neq}$ and $\Delta S^{\neq}$ are more positive than the corresponding ones determined for the reactions with the monomers. Compare, for example, $\Delta H^{\neq} = 51 \pm 8$ kJ mol^{-1} and $\Delta S^{\neq} = 3 \pm 10$ J K^{-1} mol^{-1} for the reaction with d($T_8^{s4}UT_8$) with the values of 32 ± 1 kJ mol^{-1} and -69 ± 10 J K^{-1} mol^{-1} obtained for ^{s4}UMP. A similar trend for the change in the apparent activation parameters after incorporation of the binding site into the DNA environment was also observed for reactions of the cationic Au(III) complex [25]. Thus, the activation parameters give additional support for a common contribution to the observed reactivity for these two rapidly reacting metal centers.

The reaction model outlined above allows for a resolution of the observed activation parameters into contributions from the pre-equilibrium step, Eq. (4), and the rate determining adduct formation, Eq. (5), according to $\Delta H^{\neq} = \Delta H^0_{ass} + \Delta H_2^{\neq}$ and $\Delta S^{\neq} = \Delta S^0_{ass} + \Delta S_2^{\neq}$ [62]. The $\Delta S^{\neq}$-values obtained for the oligonucleotide reactions suggest that the activation process involves an increased degree of disorder in the system during the activation process. The intramolecular reaction, Eq. (5), is the better candidate for such contribution. This reaction step most likely involves formation of a pentacoordinated transition state, where the cationic metal complex is linked to the final binding site in the center of the oligomer. It is reasonable to assume that the resulting reduction of the axial charge density of the oligomer dramatically changes its ability to attract both bulk cations and water molecules [16–21]. In addition, the flexibility of the oligomer might increase as a result of the reduction of repulsive intramolecular electrostatic interactions.

The presence of an Eigen–Wilkins type pre-equilibrium, Eq. (4), is usually found to facilitate product formation for reactions between small molecules where the steric requirements for productive encounters are few and not energetically demanding. The increased reactivity can then be regarded as being due to a reduction in the activation energy by the negative contribution from the pre-association step, in which the ΔH^0-value is often the predominant factor. For the electrostatically driven metal association with the DNA-surface however, a situation may arise where the preferred association sites could be located in such a way that the bond formation process is energetically more demanding compared to the reaction in the absence of a surrounding polymer. The observation of large and positive $\Delta H^{\neq}$-values in the present systems is perhaps not so unexpected, considering that pre-association should be favored to the phosphodiester backbone, whereas the binding sites are located on the neutral bases. The present use of the chelating ligand as coordination environment for the Pd(II) complex may also contribute to make the formation of the five-coordinated transition state more difficult in comparison to previously investigated Au(III) complexes [25].

In summary, incorporation of the non-charged ^{s4}U and ^{s6}I bases into short single-stranded DNA results in a significant increase in the rate of adduct formation with the rapidly reacting complex Pd(dien)Cl^+. A comparison of the activation parameters for the reactions of monomer systems with those for the oligomers, e.g. d(^{s4}U) and ^{s4}UMP with d($T_8^{s4}UT_8$), shows that both the activation enthalpy and activation entropy are influenced by the DNA environment. The $\Delta H^{\neq}$- and $\Delta S^{\neq}$-values are both found to be significantly more positive after incorporation of the ^{s4}U- and ^{s6}I-moieties into the middle of the single-stranded 17-mer oligonucleotide. The observed DNA-promoted effect is consequently the result of a reduced contribution from the activation entropy term which counteracts the increase of the $\Delta H^{\neq}$-value. Thus, it seems likely that changes in solvational properties and/or structure play a central role in determining the apparent reactivity of a functional group located on the surface of these single-stranded oligomers. The results highlight the need for a dynamic picture of the solution behavior of both short and extended DNA as a requirement for the correct prediction of reactivity as a function of detailed sequence.

4. Supplementary material

Observed pseudo-first-order and second-order rate constants for substitution of the chloride on [Pd(dien)Cl]$^+$ by d(^{s4}U), ^{s4}UMP, d(^{s6}I) or ^{s6}IMP as a function of temperature and pH and Eyring plot (Table S1, Fig. S1) and observed pseudo-first-order and second-order rate constants for the metallation of d($T_8^{s4}UT_8$) and d($T_8^{s6}IT_8$) by [Pd(dien)Cl]$^+$ as a function of temperature (Tables S2 and S3 and Fig. S1) (7 pages) are available from the authors.

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