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Non-uniform rate for platination of guanine-N7 located in short DNA oligomers†

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The rate for adduct formation between *cis*-[Pt(NH$_3$)(c-NH$_2$C$_6$H$_{11}$)Cl(OH$_2$)]$^+$ and guanine-N7 in d(T$_n$GT$_{16-n}$) decreases gradually from the maximum rate found for platination of the middle position towards both the 3′ and 5′ ends.

Short DNA and RNA fragments are currently of great scientific interest. Modified DNA fragments have emerged as promising candidates for the treatment of both cancers and viral infections.[1] In addition, increasing knowledge about the nature of the interactions between metal ions or naturally occurring proteins and the surface of ribonucleic acids *in vivo* allows for detailed models system studies under well defined conditions *in vitro*.[2] The use of oligonucleotides, rather than extended ribonucleic acids for such studies is, however, likely to change the magnitude of the electrostatic interactions between the oligonucleotide surface and approaching charged or dipolar species.[3–8] A goal for current work in our laboratory is to increase the understanding of how the reduced electrostatic interactions of such model systems influence the overall adduct formation process with charged anticancer active metal complexes. We here present a study that allows for a unique comparison of the kinetic influence from the DNA environment during metalation of the non-charged guanine-N7, G-N7, with that of the previously studied charged phosphorothioate group, p(S).[9,10] The obtained reaction profile reveals a less pronounced kinetic influence from the surrounding DNA, which suggests the distance from the phosphodiester backbone as a parameter of influence on the adduct formation process.

The present study has been designed to obtain detailed information concerning the magnitude of the kinetic influence from the DNA environment as a function of position of G-N7 within a given size DNA fragment. The reaction between *cis*-[Pt(NH$_3$)(c-NH$_2$C$_6$H$_{11}$)Cl(OH$_2$)]$^+$ **1** and a single-stranded 17-mer poly(dT) oligonucleotide with one guanine base at variable position was used as a model system; d(T$_n$GT$_{16-n}$), *n* = 0, 2, 4, 6, 8, 10, 12, 14, and 16. The platinum complex is considered to be the active metabolite of the orally adiministered anticancer active compound *cis*,*trans*,*cis*-[Pt(NH$_3$)(c-NH$_2$C$_6$H$_{11}$)(OC(O)CH$_3$)$_2$Cl$_2$] (JM216).[11–13] Like cisplatin, it preferentially forms intrastrand adducts between adjacent G-N7, but exhibits a reduced preference for formation of the mutagenic AG-lesion.[14,15] Determination of the rate constants for the adduct formation reactions with deoxyguanosine 5′-phosphate, d(pG), was included to illustrate the kinetic influence from the surrounding polymer. A low concentration salt buffer with [cation] = 1.0 mM was used to obtain maximum kinetic resolution between the different positions along the oligonucleotide. The kinetics were studied under pseudo-first-order conditions with a 40-fold excess of **1**. Samples were taken from the thermostated reaction mixture (pH 4.1 ± 0.1, 298 K) at different time intervals, and were analyzed by use of HPLC as described previously.[16] Observed pseudo-first-order rate constants, k_{obs}, were derived by a fit of a single-exponential function to the time dependent decrease or increase of peak areas corresponding to unplatinated and platinated oligomer respectively. During reactions with the oligomers, the concentrations of **1** and DNA were kept constant. The pseudo-first-order rate constants can thus be directly compared and used as a measure of the kinetic influence from surrounding DNA.

The observed rate constants were found to vary significantly with the location of the common G-N7 nucleophile along the oligomer d(T$_n$GT$_{16-n}$), see Fig. 1. The shape of the reaction profile shows that platination is kinetically favoured when the G-N7 site is located in the central part of the oligomer with a maximum for platination of d(T$_8$GT$_8$) with k_{obs} = 6.46 ± 0.04 s^{-1}. The platination rate decreases gradually in both the 5′- and 3′-directions, reaching a similar value at both ends, *viz.* k_{obs} = 1.9 ± 0.5 and 2.48 ± 0.13 s^{-1}, respectively. The observed reactivity of the mononucleotide d(pG) with k_{obs} = 0.27 ± 0.04 s^{-1} is *ca.* one order of magnitude slower compared with platination of the oligomers despite the larger Pt(II)-concentration used (C_{Pt} = 3.2 × 10^{-4} M).

The observed increased reactivity of purine N7 in the DNA environment, compared with the reactivity of its corresponding monomeric form,[17,18] agrees qualitatively well with previous observations made for reactions with the charged p(S)-group.[9,10,19,20] However, the present study shows clearly that the influence from the DNA environment is significantly reduced for reaction with the non-charged G-N7 when the gradient between the concentration of bulk cations and those accumulated on the DNA is increased.[3–8] For example, the second-order rate constant for adduct formation with the d(T$_8$GT$_8$) oligomer is *ca.* 50 times larger compared with that observed for the d(pG) monomer, whereas the reactivity of d(T$_8$p(S)T$_8$) exceeds that of d(Tp(S)T) with a factor of *ca.* 200 at comparable salt concentration.[10] The effect is further

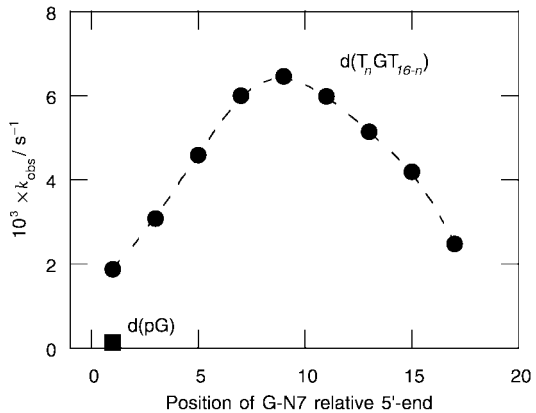

Fig. 1 Observed pseudo-first-order rate constants as a function of G-N7 position with respect to the 5′-end for reaction of **1** with d(pG), and d(T$_n$GT$_{16-n}$); *n* = 0, 2, 4, 6, 8, 10, 12, and 16, *T* = 25 °C, [cation] = 1.0 mM. For reactions with oligonucleotides: C_{Pt} = 1.6 × 10^{-4} M, [DNA] = 4.0 × 10^{-6} M, for reactions with d(pG): C_{Pt} = 3.2 × 10^{-4} M, [DNA] = 3.3 × 10^{-5} M.

† Electronic supplementary information (ESI) available: observed pseudo-first-order and apparent second-order rate constants for platination of G-N7 located in short DNA oligomers. See http://www.rsc.org/suppdata/cc/b1/b103087f/

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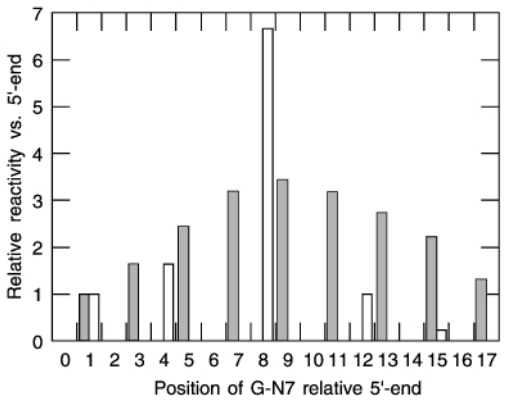

Fig. 2 Relative reactivity for adduct formation of **1** with G-N7 (hatched bars) and p(S) (open bars)[10] as a function of distance from the 5′-end of $d(T_nGT_{16-n})$ and $d(T_np(S)T_{16-n})$.

illustrated by a comparison of the reaction profiles obtained for $d(T_nGT_{16-n})$ and $d(T_np(S)T_{16-n})$, see Fig. 2. The figure reveals a similar overall tendency for a gradual increase of the reactivity towards the middle of the oligomer. Normalization of the reactivity with respect to the rate obtained for the 5′-end positions shows however that the relative change is less pronounced for formation of the platinated G-N7 moiety.

In mechanistic terms, these observations have several implications. First of all, the DNA promoted reactivity is in line with the previously suggested reaction mechanism where an electrostatically driven preequilibrium between the charged metal complex and the oligonucleotide contributes to increase the rate of adduct formation with reactive groups located on the oligomer.[9,10,19,20] Second, the gradual increase of k_{obs} towards the middle of the oligomer rules out a strict sequence dependent influence on the observed reactivity. The common platination environment of the type 5′-T_4GT_4-3′ should be large enough to provide a constant local structural and electrostatic environment around G-N7 in the centrally located positions, *i.e.* $d(T_nGT_{16-n})$; n = 4, 6, 8, 10, and 12 in the absence of other effects. Rather, the similar shape of the reaction profiles speaks in favour of a reaction model where the tendency for preaccumulation varies along the oligomer, in a fashion similar to the one calculated for short double-stranded DNA oligomers.[3,4] The less pronounced influence from such effect in the present investigation, compared with adduct formation with p(S), suggests further that the local concentration of preaccumulated cations decreases with increasing distance from the phosphodiester backbone.‡ To our knowledge, this is the first experimental investigation giving information about the corresponding variation in reactivity for a bimolecular reaction between an external, cationic metal reagent and reactive groups located on the DNA surface.

In summary, the present results support a reaction model where a varying local concentration of preassociated cationic platinum complexes significantly influences the observed reactivity during adduct formation with non-charged groups located on the DNA surface. The lower sensitivity of G-N7, compared with phosphorothioate reactions, can be explained by the larger distance between the electrostatically preferred interaction sites along the phosphodiester backbone and G-N7.[21] These results should be of importance for synthetic strategies and for a fundamental understanding of reaction mechanisms *in vivo* involving interactions between charged species and reactive groups located on small ribonucleic acid fragments or their charged antisense mimics.

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Notes and references

‡ The increased reactivity in the DNA environment can be accounted for by assuming a reaction mechanism where preaccumulation of **1** on the DNA surface contributes to the adduct formation rate by electrostatic condensation and resulting increased local concentration of **1** in the vicinity of G-N7.[10,16,20]

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