Large variation of rates for platination of phosphorothioate-containing oligonucleotides: end effects and counter ion influence

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The rate of site-specific platination of short oligonucleotides can be varied up to 500 times by a change of the bulk composition and the molecular environment surrounding the binding site.

Interactions between metal complexes and DNA are influenced by several factors. Some are related to the coordination environment and kinetic characteristics of the metal complex, others to the electrostatic and structural properties of the polymeric DNA. Improving our understanding of the mechanism for metal ion–DNA interactions is important, since DNA is believed to be the major target for many metal-based antitumour drugs.[1] In the case of biologically relevant intermediates of antitumour active *cis*-platinum complexes, several observations indicate that the location of their primary monofunctional adducts with DNA is likely to be controlled by kinetic rather than thermodynamic factors.[1–6] The aim of the present study was to further elucidate how general parameters such as location and molecular environment of a particular binding site as well as bulk composition may be utilized to obtain maximum kinetic separation between different types of adducts. The influence on reactivity originating from variations in the molecular structure surrounding the binding site was investigated by a comparison of the reactivity of $d(T_8p(S)T_8)$ with that of $d(Tp(S)T_{15})$ and d(Tp(S)T). Incorporation of a single phosphorothioate binding site has previously been shown to result in specific platination of the sulfur donor, both in the presence and absence of the naturally preferred G-N^7 binding site.[2,3,7–9] The influence of bulk composition on the rate of platination of the oligonucleotides with *cis*-$[Pt(NH_3)(NH_2C_6H_{11})Cl(OH_2)]^+$ **1**, was studied at constant pH 4.2 by a variation of the concentration of inert monovalent bulk cations in the interval 1.5 mM–0.50 M. As previously discussed, the use of the monoaqua complex **1** simplifies the reaction mechanism to that of direct substitution of the labile H_2O ligand by the nucleophilic sulfur atom of the phosphorothioate containing oligonucleotides.[3]

The cationic monoaqua complex **1** is a weak acid with pK_a 6.4 ± 0.2, and its reactivity towards phosphorothioates has previously been shown to decrease with increasing bulk pH in the range 5–8.[3] This decrease in reactivity was rationalized in terms of an increase in concentration of the less reactive monohydroxo complex, *cis*-$[Pt(NH_3)(NH_2C_6H_{11})Cl(OH)]$ **2**. Such decrease has been observed also for other model systems for interactions of mono-aquated platinum complexes with DNA.[10,11] The use of binding sites located on a negatively charged oligonucleotide makes the mechanistic interpretation of rate variations as a function of pH more complicated in the present systems than in the case of reactions between small molecules, however. The non-selective electrostatic attraction of cations onto the surface of the polymer that can be expected to take place here,[12–14] implies that not only cationic reactants but also oxonium ions may accumulate in its vicinity.[15–17] In a situation where the distribution between **1** and **2** is sensitive towards small variations of pH, it may thus be possible that an observed difference in reactivity between adduct formation on the polymer and the corresponding reaction in absence of surrounding bases could, at least partially, be the result of a local pH change. The present study made at pH 4.2 minimizes such contributions since at this pH the equilibrium distribution between **1** and **2** is affected to a negligible extent by local changes of the oxonium ion concentration. The use of a diluted buffer allowed for pH control during the reaction with a minimum of interference from competitive reactions with buffer anions.[18]† The reactions of **1** with the oligonucleotides were studied under pseudo-first-order conditions with Pt^{II} in *ca.* 10-fold excess compared to the concentration of oligomers by use of HPLC. The kinetic parameters were evaluated at 298 K from the time-dependent changes in integrated HPLC peak areas of unplatinated and platinated oligonucleotides, respectively (Supplementary data, Fig. S1).‡ Both the decay of reactants and formation of products were well described by single exponentials (Supplementary data, Fig. S2). The observed pseudo-first-order rate constants, k_{obs}, were linearly dependent on the total concentration of Pt^{II} (Supplementary data, Table S1, and Fig. S3).

As shown in Fig. 1, the apparent second-order rate constants, $k_{2,app}$,§ for platination of the oligonucleotides exhibit a common trend for $[Na^+, K^+] \leqslant 0.3$ M with a monotonous increase in reactivity as a function of decreasing bulk cation concentration. In addition, a relative reactivity order of $k_{2,app}(d(T_8p(S)T_8)) > k_{2,app}(d(Tp(S)T_{15})) > k_{2,app}(d(Tp(S)T))$ is maintained throughout the whole concentration range investigated. There are striking differences in reactivity behaviour, however. First of all, the limiting rate constants obtained for $[Na^+, K^+] > 0.3$ M are significantly different for the three types of DNA: 5.7 ± 1.5, 0.87 ± 0.15 and 0.43 ± 0.05 $dm^3 mol^{-1} s^{-1}$ for $d(T_8p(S)T_8)$, $d(Tp(S)T_{15})$ and d(Tp(S)T), respectively. Secondly, the response to decreasing salt concentration exhibits different characteristics with an increase in reactivity that is most pronounced, both in absolute and relative terms, for platination of $d(T_8p(S)T_8)$. The maximum second-order rate constant for the latter reaction was determined as 200 ± 40 $dm^3 mol^{-1} s^{-1}$ at the lowest salt concentration investigated, $[Na^+, K^+]$ = 1.5 mM. At this concentration of bulk cations the rate constant for

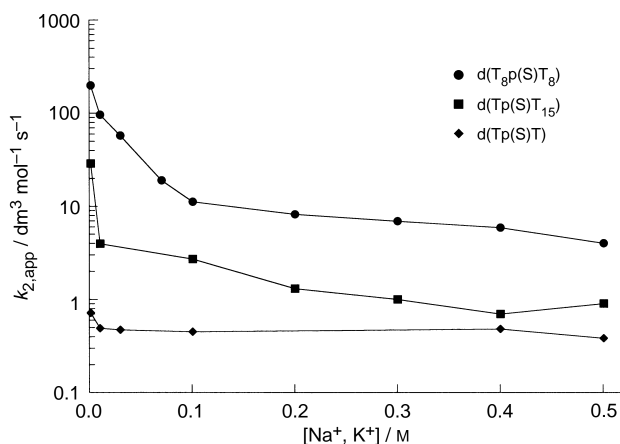

Fig. 1 Semilogarithmic plot of the apparent second-order rate constant *vs.* the concentration of monovalent bulk cations for reaction of **1** with (●) $d(T_8p(S)T_8)$, (■) $d(Tp(S)T_{15})$ and (◆) d(Tp(S)T), at pH 4.2 and 25 °C

platination of d(Tp(S)T_{15}) was found to be 30 ± 10 $dm^3\ mol^{-1}\ s^{-1}$. With respect to the rate of platination of d(Tp(S)T), $k_{2,app}$ = 1.0 ± 0.5 $dm^3\ mol^{-1}\ s^{-1}$ at [Na^+, K^+] = 1.5 mM, these rate constants thus represent a 200- and 30-fold rate increase for d(T_8p(S)T_8) and d(Tp(S)T_{15}), respectively.

These results clearly demonstrate that bulk conditions as well as the molecular environment of the phosphorothioate binding site have a large influence on the rate of platination also of short single-stranded oligonucleotides which lack the typical polyelectrolyte behaviour of extended DNA.[14,19–21] It is noteworthy, that although the absolute value of the rate constants may be changed by orders of magnitude by a variation of the concentration of monovalent cations in the bulk, such a variation does not affect the relative reactivity order for formation of the different types of adducts. Surprisingly, the rate of platination of d(Tp(S)T_{15}) exhibits reaction characteristics intermediate between those of d(T_8p(S)T_8) and d(Tp(S)T). This observation suggests that association of the cationic metal complex with the polymer prior to adduct formation is of importance for the reaction mechanism also for platination of the end position where the polyelectrolyte effect can be expected to be reduced to a minimum.[19–21] The present study, in which pH effects can be excluded from contributing to the rate increase, indicates that rate accelerations similar to those observed for freely diffusing species[17] may be obtained also for reactions where the rate determining step involves formation of a covalent bond with donor atoms located on the charged phosphodiester backbone of the DNA. These findings thus give strong support for a previously suggested reaction mechanism for platination of oligonucleotides comprising preassociation of metal complex onto the oligomer followed by rapid diffusion along the contour length of the polymer to the kinetically preferred binding site.[3] In addition to the impact of this mechanism on biological systems, awareness of its large influence on reactivity, as exemplified here by a maximum variation of the platination rate between d(T_8p(S)T_8) and d(Tp(S)T) by a factor of *ca.* 500, should also be of importance for antisense strategies using for example cross-linking reagents, as well as for synthetic chemistry.

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Footnotes and References

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† [$HC_8H_4O_4^-$] = 0.50 mM, pH = 4.2 adjusted with $HClO_4$.
‡ Available upon request from the authors.
§ Obtained as $k_{obs}/[Pt^{II}]_{tot}$.

1 See for example: *Metal Ions in Biological System*, ed. A. Sigel and H. Sigel, Marcel Dekker, New York, 1996, vol. 32.
2 S. K. C. Elmroth and S. J. Lippard, *J. Am. Chem. Soc.*, 1994, **116**, 3633.
3 S. K. C. Elmroth and S. J. Lippard, *Inorg. Chem.*, 1995, **34**, 5234.
4 F. Gonnet, F. Reeder, J. Kozelka and J.-C. Chottard, *Inorg. Chem.*, 1996, **35**, 1653.
5 F. Reeder, F. Gonnet, J. Kozelka and J.-C. Chottard, *Chem. Eur. J.*, 1996, **2**, 1068.
6 S. J. Berners-Price, K. J. Barnham, U. Frey and P. J. Sadler, *Chem. Eur. J.*, 1996, **2**, 1283.
7 K. G. Strothkamp and S. J. Lippard, *Proc. Natl. Acad. Sci. USA*, 1976, **73**, 2536.
8 D. J. Szalda, F. Eckstein, H. Sternbach and S. J. Lippard, *J. Inorg. Biochem.*, 1979, **11**, 279.
9 B. C. F. Chu and L. E. Orgel, *Nucl. Acids Res.*, 1990, **18**, 5163.
10 J. Arpalahti and P. Lehikoinen, *Inorg. Chem.*, 1990, **29**, 2564.
11 M. Mikola, P. Oksman and J. Arpalahti, *J. Chem. Soc., Dalton Trans.*, 1996, 3101.
12 G. S. Manning, *Quart. Rev. Biophys. II*, 1978, **2**, 179.
13 A. Pullman and B. Pullman, *Quart. Rev. Biophys.*, 1981, **14**, 289.
14 C. F. Anderson and M. T. Record, Jr., *Annu. Rev. Phys. Chem.*, 1995, **46**, 657.
15 G. Lamm and G. R. Pack, *Proc. Natl. Acad. Sci. USA*, 1990, **87**, 9033.
16 S. Hanlon, L. Wong and G. R. Pack, *Biophys. J.*, 1996, **72**, 291.
17 G. R. Pack and L. Wong, *Chem. Phys.*, 1996, **204**, 279.
18 J. E. Teggins, K. W. Lee, J. M. Baker and E. D. Smith, *J. Coord. Chem.*, 1971, **1**, 215.
19 M. C. Olmsted, C. F. Anderson and M. T. Record, Jr., *Proc. Natl. Acad. Sci. USA*, 1989, **86**, 7766.
20 M. O. Fenley, G. S. Manning and W. K. Olson, *Biopolymers*, 1990, **30**, 1191.
21 V. M. Stein, J. P. Bond, M. W. Capp, C. F. Anderson and M. T. Record, Jr., *Biophys. J.*, 1995, **68**, 1063.

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