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# Entropy Contributions to DNA-Promoted and DNA-Retarded Chemistry Revealed by Reactions of Gold(III) Complexes with Thione-Containing Nucleosides and Oligonucleotides

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Interactions of charged metal complexes with DNA are influenced by the anionic nature of the polynucleic acid. The local concentration of cations on the surface of the polymer is increased compared to that of the bulk solution, whereas anions are effectively excluded.[1−6] The resulting compartmentalization of charged species has implications in the rate at which functional groups on the DNA are modified. For example, formation of covalent adducts with cationic metabolites of anticancer active Pt(II) complexes has been shown to be favored by the DNA environment.[7−11] Our current work seeks a fundamental mechanistic understanding of factors contributing to such behavior. The present study is focused on evaluation of the influence on reactivity due to the global polyanionic properties of short single-stranded oligonucleotides compared to that associated with the local environment of the target site on the individual bases. Two oppositely charged gold(III) complexes and their interactions with thione-containing single-stranded oligonucleotides, $d(T_8XT_8)$ X = $d(^{s6}I)$ or $d(^{s4}U)$,[12,13] have been utilized for these initial studies (Chart 1). The spectral features of the thiones have previously been shown to serve as useful UV/vis probes for metalation reactions.[14−17] The metal complexes used, *trans*-$[Au(NH_3)_2Cl_2]^+$ (**1**) and *trans*-$[Au(CN)_2Cl_2]^-$ (**2**), have molecular dimensions and reaction characteristics similar to those of platinum(II) complexes.[18] Their reactivity exceeds that of Pt(II) by about 6 orders of magnitude, and the use of these complexes thus adds a kinetics constraint on the suggested preassociation step[7,8,11] to obtain DNA-promoted reactivity with the cationic reagent.

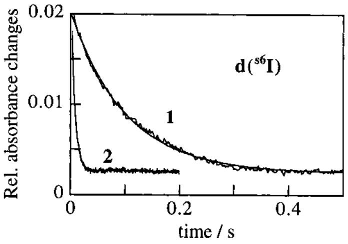
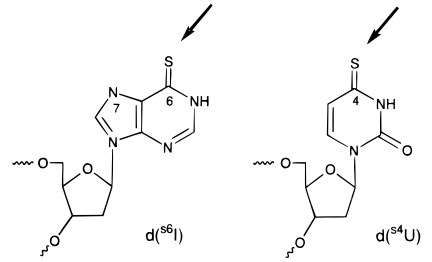


**Figure 1.** Typical kinetic traces at 322 nm for the reaction of $d(^{s6}I)$ with **1** and **2** at 25.0 °C and pH 4.0. The solid lines represent the least-squares fit of a single-exponential function to the experimental data; $C_{Au(1)} = 1.7 \times 10^{-4}$ M and $[d(^{s6}I)] = 1.9 \times 10^{-6}$ M, $C_{Au(2)} = 5.3 \times 10^{-5}$ M and $[d(^{s6}I)] = 2.4 \times 10^{-6}$ M, pH 4.0 with $KHC_8H_4O_4$, $[Cl^-] = 5.0 \times 10^{-3}$ M, $I = 0.11$ M.

## Chart 1



The kinetics for the interaction of the two gold(III) complexes with the nucleosides and oligonucleotides was studied with at least a 10-fold excess concentration of metal complex over thione. The pH of the reaction mixture was kept at 4.0 with phthalate buffer[19] containing sodium chloride to prevent hydrolysis of the metal complexes. Reactions were initiated by the mixing of equal amounts of thermostated reagent solutions directly in the stopped-flow instrument. The kinetics were monitored by the use of the absorbance decrease associated with the disappearance of free thione in solution, which occurred with a maximum intensity change at ca. 322 nm for $d(^{s6}I)$ and 332 nm for $d(^{s4}U)$. The kinetics traces were well-described by single exponentials under these conditions (Figure 1). The first-order rate constants, $k_{obsd}$, exhibited a linear dependence on the concentration of metal complex throughout the temperature range investigated. A least-squares analysis of the dependence of $k_{obsd}$ on total concentration of **1** or **2** resulted in nonsignificant intercepts and apparent second-order rate constants $k_{2,app}$ that could be determined directly from the slopes or as $k_{obsd}/C_{Au}$. An experimental rate law could thus be deduced according to eq 1 . The rate law indicates a common

$$-d[\text{thione}]/dt = k_{2,app}\,[\text{Au(III)}][\text{thione}] \qquad (1)$$

reaction mechanism for interaction with the thione sites in both the nucleosides and oligonucleotides, without influence from the reverse or solvent path reactions of the metal complexes. A summary of the values of $k_{2,app}$ and the corresponding activation parameters is given in Table 1.

The inherent reactivity of the two types of thione moieties is illustrated by the second-order rate constants for reactions of the noncharged nucleosides $d(^{s6}I)$ and $d(^{s4}U)$ with **1** and **2** (Table 1).

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**Table 1.** Second-Order Rate Constants at 25 °C and Activation Parameters for Reaction of Thione-Containing Nucleosides and Oligonucleotides with **1** and **2**[a]

| | **1** + L | | | **2** + L | | |
|---|---|---|---|---|---|---|
| L | $10^{-4} \times k_{2,\mathrm{app}}$/M$^{-1}$ s$^{-1}$ | $\Delta H^{\ddagger}$/kJ mol$^{-1}$ | $\Delta S^{\ddagger}$/J K$^{-1}$ mol$^{-1}$ | $10^{-5} \times k_{2,\mathrm{app}}$/M$^{-1}$ s$^{-1}$ | $\Delta H^{\ddagger}$/kJ mol$^{-1}$ | $\Delta S^{\ddagger}$/J K$^{-1}$ mol$^{-1}$ |
| d($^{s6}$I) | 5.5 ± 0.9 | 34 ± 3 | −38 ± 10 | 19 ± 1 | 26 ± 1 | −37 ± 3 |
| d($\mathrm{T_8{}^{s6}IT_8}$) | 30 ± 5 | 34 ± 2 | −31 ± 6 | 5.7 ± 1.0 | 15.5 ± 1.5 | −83 ± 5 |
| d($^{s4}$U)[b] | 1.6 ± 0.1 | 21.7 ± 1.1 | −90 ± 4 | 16 ± 1 | 16 ± 2 | −72 ± 6 |
| d($\mathrm{T_8{}^{s4}UT_8}$) | 3.8 ± 0.2 | 36 ± 3 | −30 ± 10 | 7.9 ±1.0 | 7.5 ± 0.7 | −108 ± 3 |

[a] Errors for activation parameters given as one standard error. [b] Reference 17.

The rate constants obtained for reaction with **2** are 1−2 orders of magnitude larger than those for **1**. The higher reactivity of **2** can be ascribed to the reduction of the $\Delta H^{\ddagger}$-value by 8 and 6 kJ mol$^{-1}$ for reaction with d($^{s6}$I) and d($^{s4}$U), respectively. A common trend in reactivity is observed for the two metal complexes, favoring reaction with d($^{s6}$I) over d($^{s4}$U). The larger rate constant obtained for nucleophilic displacement of the chloride ligand by d($^{s6}$I) is an effect of the relatively small activation entropy, ca −38 J K$^{-1}$ mol$^{-1}$ for reaction with both **1** and **2**. In the reactions with d($^{s4}$U), significantly more negative activation entropies are found, $\Delta S^{\ddagger} = -90 \pm 4$ J K$^{-1}$ mol$^{-1}$ for reaction with **1** and $\Delta S^{\ddagger} = -72 \pm 6$ J K$^{-1}$ mol$^{-1}$ for **2**, which effectively counterbalance the more favorable activation enthalpy term obtained for these reactions.

Contributions to the reactivity from charge interactions and more demanding stereochemistry are introduced after incorporation of the nucleosides into the oligonucleotide fragments. With nucleoside reactivity as a reference, the second-order rate constants indicate that charge interactions dominate the apparent reactivity for metalation of the oligonucleotides. Consequently, reactions with **1** are promoted in the oligonucleotide environment by a factor of 2−5, with the larger rate increase obtained for d($\mathrm{T_8{}^{s6}IT_8}$). In contrast, the reactions of **2** are retarded by a factor of 2−3 compared to the reaction with the nucleoside.

The different response of **1** and **2** to the DNA environment is further confirmed by an inspection of the activation parameters. In mechanistic terms, the more simple reaction should be exhibited with the anionic complex **2**. The repulsive forces between **2** and d($\mathrm{T_8XT_8}$) where X = d($^{s6}$I) or d($^{s4}$U) are likely to prevent close interactions with the oligonucleotide fragment other than those involved in direct formation of the thione adduct. Surprisingly, the $\Delta H^{\ddagger}$-values obtained for reaction of **2** with the oligonucleotides are significantly lower (ca 10 kJ mol$^{-1}$) than those with the nucleoside. The small $\Delta H^{\ddagger}$-values indicate that the bond formation involved in the rate-determining step is actually facilitated in the DNA environment despite the large steric bulk of the oligomer. Thus, the apparent reduction in rate observed for reaction with **2** at 25 °C is solely an effect of the unfavorable $\Delta S^{\ddagger}$-term. It is noteworthy that the magnitude of the $\Delta S^{\ddagger}$-values obtained with the oligomers are significantly more negative than those for the corresponding nucleosides, −83 ± 5 J K$^{-1}$ mol$^{-1}$ for the reaction with d($\mathrm{T_8{}^{s6}IT_8}$) and −108 ± 3 J K$^{-1}$ mol$^{-1}$ for the reaction with d($\mathrm{T_8{}^{s4}UT_8}$). The decrease of the activation entropy by ca. 40 J K$^{-1}$ mol$^{-1}$ obtained for both types of thione sites shows that the addition of the single negative charge in the middle of the 17-mer oligonucleotide induces similar entropy changes for the two oligomers. Such common behavior could, for example, involve additional accumulation of bulk cations on the surface of the oligomer as a result of the charge formation process and/or reduced degrees of freedom in the polymer chain during the formation of the transition state.

In the reaction with the cationic complex **1**, both a direct reaction and one operating via a nonspecific preaccumulation of **1** on the surface of the oligomer may contribute to adduct formation. Compared to the reactions with the monomers, an additional positive contribution to the activation entropy is expected for both types of mechanisms as a result of the charge neutralization and concomitant loss of hydration and/or counterions in the DNA vicinity during the adduct formation step. Inspection of the data in Table 1 shows that incorporation of d($^{s4}$U) into the DNA environment results in changes of the $\Delta S^{\ddagger}$-values in agreement with these expectations with an increase in $\Delta S^{\ddagger}$ from −90 to −30 J K$^{-1}$ mol$^{-1}$. A similar trend is also observed for d($^{s6}$I) even if the change from −38 to −31 J K$^{-1}$ mol$^{-1}$ falls within the error limits. The larger sensitivity exhibited by the cationic metal complex **1** toward the local environment is also illustrated by a comparison of the $\Delta H^{\ddagger}$-value in the absence and presence of surrounding DNA-bases where only incorporation of d($^{s4}$U) into d($\mathrm{T_8{}^{s4}UT_8}$) results in a significant change. It should be noted that the larger $\Delta H^{\ddagger}$-value observed in the DNA environments suggests contributions to the reaction mechanism other than a simple preequilibrium of outer-sphere type, since the presence of such would contribute to decrease the $\Delta H^{\ddagger}$-value.[20,21]

To conclude, we have found that interactions of gold(III) complexes with single-stranded 17-mer oligonucleotides exhibit both DNA-promoted and DNA-retarded reactivity depending on the charge of the metal complex. The apparent correlation between the nature of the electrostatic interactions, as either attractive or repulsive, and the change in reactivity after incorporation into the DNA environment is shown to be primarily an effect of the activation entropy term. These findings are in line with a polyelectrolyte-like behavior of the oligonucleotides, and they illustrate the importance of relatively small charge interactions for the apparent reactivity of the functional groups located on the oligomer. In addition to such general effects, a more subtle influence on the reactivity from the local environment of the target site is observed. Thus, the DNA-promoted reactivity found for cationic reactants in the presence of DNA oligomers, which already has been suggested to be an important factor for the antitumor activity of cisplatin,[7,8,11] is likely to be applicable also to significantly more rapid processes involving positively charged reactants and formation of their covalent DNA-adducts.

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**Supporting Information Available:** Spectral changes as a function of time for reactions of d($\mathrm{T_8{}^{s4}UT_8}$) with **2**, observed first-order rate constants for reactions of d($^{s6}$I), d($^{s4}$U), d($\mathrm{T_8{}^{s6}IT_8}$), and d($\mathrm{T_8{}^{s4}UT_8}$) with **1** and **2** as a function of excess gold(III) concentration, apparent second-order rate constants for reactions of d($^{s6}$I), d($\mathrm{T_8{}^{s6}IT_8}$), and d($\mathrm{T_8{}^{s4}UT_8}$) with **1** and **2** as a function of temperature and corresponding Eyring plots (5 pages, print/PDF). See any current masthead page for ordering information and Web access instructions.

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