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Kinetics of interaction of the thione unit in 4-thio-2′-deoxyuridine and 4-thiouridine 5′-monophosphate with rapidly reacting gold(III) complexes ‡

Anna Ericson,[a] John C. Arthur,[b] Robert S. Coleman,[c] Lars I. Elding[a] and Sofi K. C. Elmroth*,†,[a]

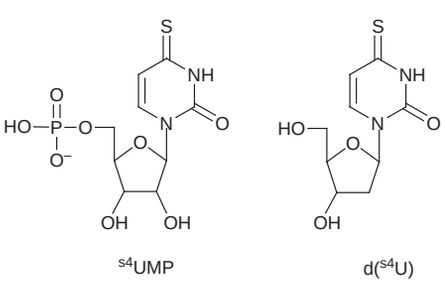
[a] *Inorganic Chemistry 1, Chemical Center, Lund University, P. O. Box 124, S-221 00 Lund, Sweden*
[b] *Department of Chemistry and Biochemistry, University of South Carolina, Columbia, SC 29208, USA*
[c] *Department of Chemistry, The Ohio State University, 100 West 18th Avenue, Columbus, OH 43210-1185, USA*

Reactions of 4-thio-2′-deoxyuridine d(^{s4}U) and 4-thiouridine 5′-monophosphate ^{s4}UMP with *trans*-$[AuY_2Cl_2]^{+/-}$ (Y = CN^- or NH_3) have been investigated by conventional and high-pressure stopped-flow spectrophotometry in aqueous solution with 2.9 ⩽ pH ⩽ 7.0. For a given combination of metal complex and sulfur donor the observed second-order rate constants decreased with increasing pH. In the pH range 2.9 to 5.5 this decrease is mainly due to displacement of the pH-dependent equilibrium *trans*-$[AuY_2Cl_2]^{+/-} + H_2O \rightleftharpoons$ *trans*-$[AuY_2Cl(OH)]^{+/-} + Cl^- + H^+$ and formation of an increasing fraction of the less reactive gold(III) hydroxo complex. The activation parameters $\Delta H^‡$, $\Delta S^‡$ and $\Delta V^‡$ at pH 4.0 for substitution of chloride for thione at *trans*-$[AuY_2Cl_2]^{+/-}$ indicate an associative interchange process. When the thione-containing nucleotide reacts with the cation, steric and electrostatic interactions between the metal complex and the phosphate group oppose each other and the rate of the reaction of *trans*-$[Au(NH_3)_2Cl_2]^+$ with ^{s4}UMP is approximately the same as with d(^{s4}U). Thus, the electrostatic outer-sphere interaction with a single phosphate group adjacent to the kinetically preferred binding site is not sufficient to induce a reactivity that significantly exceeds that of direct interaction between the thione and the rapidly reacting gold(III) centre. In contrast, reaction of *trans*-$[Au(CN)_2Cl_2]^-$ with ^{s4}UMP is significantly slower than with d(^{s4}U), most likely due to the combined effect of electrostatic repulsion and steric blocking.

Interactions of metal complexes with polynucleic acids often take place in a non-random fashion.[1–4] The polyelectrolyte properties of the polymer selectively favour reactions of cations with donor atoms on the surface,[5–10] and its chiral structure imposes steric requirements on these interactions.[3,11] In addition, local variations of hydrophilicity and the sequence-dependent electrostatic potential are expected to influence the site of final binding of the metal centre.[12,13] The need for a better and more general understanding of the principles governing these interactions is illustrated for example by the two most common adducts of *cis*-$[Pt(NH_3)_2Cl_2]$ (*cis*-platin) with DNA, the GpG and ApG adducts.[14] The preference of platinum for these binding sites has been suggested to be part due to kinetic factors, possibly as a result of the favourable electrostatic potential created around these sequences.[12] The sensitivity of biological systems towards the various adducts affords an additional challenge for optimised kinetic control of formation of the GpG adduct. This adduct is today believed to be responsible for the anticancer activity of *cis*-platin,[4,15] whereas the ApG adduct is highly mutagenic.[16]

The interpretation of kinetic data for bonding of metal complexes to extended polynucleic acid faces a common problem; that of bonding to multiple sites with similar rate constants. A detection method which selectively allows for evaluation of the kinetics for binding to a given local sequence, also under conditions where competing metallation of nearby bases is likely to occur, is thus needed. The goal of the present work has been to evaluate the usefulness of the 4-thiouridine moiety for this purpose and further to study the relative importance of electrostatic and steric factors on the reactions between metal complexes and nucleic acid fragments.

The thione unit, which is naturally present in *Escherichia coli* tRNAs,[17] has been shown to interact specifically with metal centres like Hg^{II} and Tl^{III}.[18–20] In the present work the reactions of the deoxyribonucleoside and ribonucleotide of 4-thiouridine have been studied. Two gold(III) compounds with different ionic charges were used as model complexes, since these are expected to have a high kinetic preference for the thione unit, compared to other potential donor atoms on the nucleobases.[21] In addition, the spectral features of the unique sulfur donor in the 4-thiouridine moiety makes it useful as a specific probe of metallation at this particular binding site.[22,23] Detection and evaluation of the kinetic influence from the local environment surrounding the binding site, *i.e.* the phosphate group of 4-thiouridine 5′-monophosphate (^{s4}UMP), is thus facilitated compared to studies of more common nucleobases.



† E-Mail: Sofi.Elmroth@inorg.lu.se
‡ *Supplementary data available*: rate constants under various conditions. For direct electronic access see http://www.rsc.org/suppdata/dt/1998/1687/, otherwise available from BLDSC (No. SUP 57364, 10 pp.) or the RSC Library. See Instructions for Authors, 1998, Issue 1 (http://www.rsc.org/dalton).

Experimental

Chemicals and solutions

Buffer solutions were prepared from aqueous stock solutions of potassium hydrogenphthalate $KHC_8H_4O_4$ (Baker, AR), NaH_2PO_4 (Sigma, RG), $HClO_4$ (Merck, pa), HCl (Merck, titrisol) and NaOH (Eka, pa) by slight modification of literature procedures,[24] to maintain a constant ionic strength of 0.11 mol dm^{-3} in all experiments. Buffers below pH 5.5 were made by addition of $HClO_4$ (pH 2.9), HCl (pH 4.0) or NaOH (pH 5.0 and 5.5) to a stock solution of $KHC_8H_4O_4$. Above pH 6.0 the buffers were prepared from NaH_2PO_4 and the pH was adjusted by addition of NaOH; HCl or NaCl (Merck, pa) was added to give a final chloride concentration of at least 5 mmol dm^{-3} in the solutions. Water was doubly distilled from quartz.

The salt $K[Au(CN)_2Cl_2]\cdot H_2O$ was synthesized by oxidation of $K[Au(CN)_2]$ (Degussa) according to the literature,[25] $[Au(NH_3)_2Cl_2]Cl\cdot 0.33H_2O$ from $[Au(NH_3)_4][NO_3]_3$ with $HAuCl_4\cdot xH_2O$ (Degussa) as the starting material.[26,27] Stock solutions were prepared by dissolving a weighed amount of $K[Au(CN)_2Cl_2]\cdot H_2O$ or $[Au(NH_3)_2Cl_2]Cl\cdot 0.33H_2O$ in a buffered solution containing at least 10 mmol dm^{-3} of chloride to suppress the formation of phosphate, phthalate and aqua complexes. Estimated stability constants for oxygen-donor complexes indicate that the amount of phosphate or phthalate complex of gold(III) in the solutions will be negligible under the experimental conditions used.[21,28] Stock solutions of gold(III) in buffer were prepared directly before use.

4-Thio-2′-deoxyuridine d(^{s4}U) was prepared as described before.[29,30] Stock solutions of d(^{s4}U) and 4-thiouridine 5′-monophosphate (Sigma) were prepared by dissolving a weighed amount of d(^{s4}U) or ^{s4}UMP in buffer. The concentration was determined spectrophotometrically at 332 nm.[22] Stock solutions of d(^{s4}U) and ^{s4}UMP were stable when kept refrigerated in the dark.

Apparatus

Spectra were recorded by use of a Milton Roy 3000 diode-array spectrophotometer and thermostatted 1.00 cm Quartz Suprasil cells. Time-resolved spectra and kinetics at ambient pressure (10–50 °C) were recorded with an Applied Photophysics Biosequential SX-17MV, stopped-flow ASVD spectrofluorimeter. Kinetic runs were evaluated by use of an on-line least-squares minimising program, SX-17MV.[31] The pressure dependence was studied at 25.0 °C by use of a Hi-Tech HPSF-56 high-pressure stopped-flow spectrophotometer,[32] and data were evaluated by an on-line OLIS computer program.[33] Proton NMR spectra were recorded on a Varian Unity 300 MHz spectrometer.

Kinetics

All reactions were monitored with at least a ten-fold excess of gold(III). Reactions were started by mixing equal volumes of solutions containing gold(III) complex with solutions of d(^{s4}U) or ^{s4}UMP directly in the stopped-flow instrument. They were monitored using the decrease in absorbance at 332 nm, corresponding to the decrease of free d(^{s4}U) or ^{s4}UMP in solution. Under those conditions the kinetic traces were well described by single exponentials. Reported pseudo-first-order rate constants, k_{obs}, were determined as an average of at least five independent kinetic runs. Second-order rate constants were obtained from a fit of a straight line to the pseudo-first-order rate constants *vs.* the total concentrations of the gold(III) complex using a least-squares minimisation routine. Enthalpies and entropies of activation were obtained by a fit of the Eyring equation to the natural logarithm of the second-order rate constant, $\ln(k/T)$ *vs.* $1/T$. Volumes of activation were derived by a fit of equation (1) to the rate constants at variable pressure.

$$\ln k = \ln k_0 - \Delta V^{\ddagger} P/(RT) \quad (1)$$

Here, k_0 denotes the pseudo-first-order rate constant at zero pressure and 25.0 °C.

Results and Discussion

UV/VIS spectra and stoichiometry

The UV/VIS spectra of *trans*-$[Au(CN)_2Cl_2]^-$, *trans*-$[Au(NH_3)_2Cl_2]^+$ and 4-thiouridine have been published previously.[22,34,35] Addition of an excess of *trans*-$[AuY_2Cl_2]^{+/-}$ (Y = CN^- or NH_3) to a solution of d(^{s4}U) or ^{s4}UMP results in disappearance of the absorbance maximum associated with the sulfur moiety in the nucleobase at 332 nm, and a parallel increase with a maximum intensity change at *ca.* 300 nm (SUP 57364). These initial and rapid absorbance changes occur at a wavelength similar to what previously has been observed for reactions between gold(III) complexes and sulfur donors like Me_2S or SCN^-.[36–38] They can be interpreted as due to substitution of chloride for thiouridine in the co-ordination sphere of Au^{III}.[36–38] In the timescale of the reactions studied, no subsequent redox processes were observed. The stoichiometry for the reaction between *trans*-$[Au(NH_3)_2Cl_2]^+$ and ^{s4}UMP was determined from the decrease of absorbance at 217 nm for a series of equilibrated solutions with a constant five-fold excess concentration of complex and increasing concentrations of ^{s4}UMP, indicating a stoichiometric ratio of Au : ^{s4}UMP of 1 : 1.1 ± 0.1 (SUP 57364). The stoichiometry for the other three reactions studied was assumed to be the same.

^{1}H NMR spectra

Proton NMR spectra were obtained for a *ca.* 10 mmol dm^3 solution of d(^{s4}U) in D_2O at ambient temperature (D_2O, 299.780 MHz): δ 7.79 (d, 1 H, J = 7.6, C6–H), 6.63 (d, 1 H, J = 7.6, C5–H), 6.28 (t, 1 H, J = 6.5 Hz, Cl′–H), 4.50 (m, 1 H, C3′–H), 4.13 (m, 1 H, C4′–H), 3.85 (m, 2 H, C5′–H) and 2.47 (m, 2 H, C2′–H). Addition of 1 molar equivalent of *trans*-$[Au(CN)_2Cl_2]^-$ to this solution resulted in a instantaneous and quantitative conversion of the signals originating from unchanged d(^{s4}U) to reaction products (D_2O, 299.780 MHz): δ 8.40 (d, 1 H, J = 7.1, C6–H), 7.05 (d, 1 H, J = 7.1, C5–H), 6.23 (t, 1 H, J = 6.5 Hz, Cl′–H), 4.46 (m, 1 H, C3′–H), 4.20 (m, 1 H, C4′–H), 3.90 (m, 1 H, C5′–H), 3.82 (m, 1 H, C5′–H), 2.66 (m, 1 H, C2′–H) and 2.40 (m, 1 H, C2′–H). Comparison of these spectra show that in particular the signals originating from the C6 and C5 protons are significantly perturbed after addition of the gold(III) complex, consistent with metal co-ordination at the sulfur site.

Reactivity of the thione in d(^{s4}U) and ^{s4}UMP

Examples of kinetic traces for the reactions of *trans*-$[Au(CN)_2Cl_2]^-$ with d(^{s4}U) and of *trans*-$[Au(NH_3)_2Cl_2]^+$ with ^{s4}UMP are given in Fig. 1. The reaction has a half-life between 5 and 500 ms, depending on the pH and the nature and total concentration of the gold(III) complex. At constant pH a linear dependence was observed between the excess concentration of gold(III) and the corresponding pseudo-first-order rate constants, *cf.* Fig. 2. There are no significant intercepts for any of the four systems investigated, indicating that contributions from a solvent path or a reverse reaction are negligible.[21,39] The kinetics can thus be interpreted in terms of a simple one-step substitution reaction according to equation (2), where k denotes

$$trans\text{-}[AuY_2Cl_2]^{+/-} + L \xrightarrow{k} trans\text{-}[AuY_2Cl(L)]^{+/0/-} + Cl^- \quad (2)$$

the second-order rate constant, Y = CN^- or NH_3 and L = d(^{s4}U) or ^{s4}UMP, compare also stoichiometry discussed above.

Table 1 Second-order rate constants for the reactions of gold(III) complexes with d(^{s4}U) and ^{s4}UMP at different pH

	10^{-5} k^a/dm^3 mol^{-1} s^{-1}			
	trans-$[Au(CN)_2Cl_2]^-$		*trans*-$[Au(NH_3)_2Cl_2]^+$	
pH	d(^{s4}U)	^{s4}UMP^b	d(^{s4}U)	^{s4}UMP
2.9		9.1 ± 0.9		
		10.4 ± 0.9^c		
4.0	16 ± 1.5	9.2 ± 0.6	0.16 ± 0.01	0.16 ± 0.02
4.5				0.14 ± 0.01
5.0		4.3 ± 0.1	0.11 ± 0.01	0.093 ± 0.009
5.0		8.3 ± 0.1^d		
5.5	7.3 ± 0.3			0.066 ± 0.006
6.0		0.78 ± 0.01		
7.0	(2.1 ± 0.1) × 10^{-2}	(6.7 ± 0.6) × 10^{-3}		

^{a} $[Cl^-] = 5 \times 10^{-3}$ mol dm^{-3} unless otherwise stated. ^{b} Calculations based on the base hydrolysis equilibrium using FACSIMILE[42] and available equilibrium data[40,41] gave the following results: $k = 9.9 \times 10^5$ dm^3 mol^{-1} s^{-1} for pH 2.9, 10.1×10^5 dm^3 mol^{-1} s^{-1} for pH 4.0 and 4.9×10^5 dm^3 mol^{-1} s^{-1} for pH 5.0. ^{c} $[Cl^-] = 0.055$ mol dm^{-3}. ^{d} $[Cl^-] = 0.050$ mol dm^{-3}, see SUP 57364 for more detailed information concerning the chloride-ion dependence.

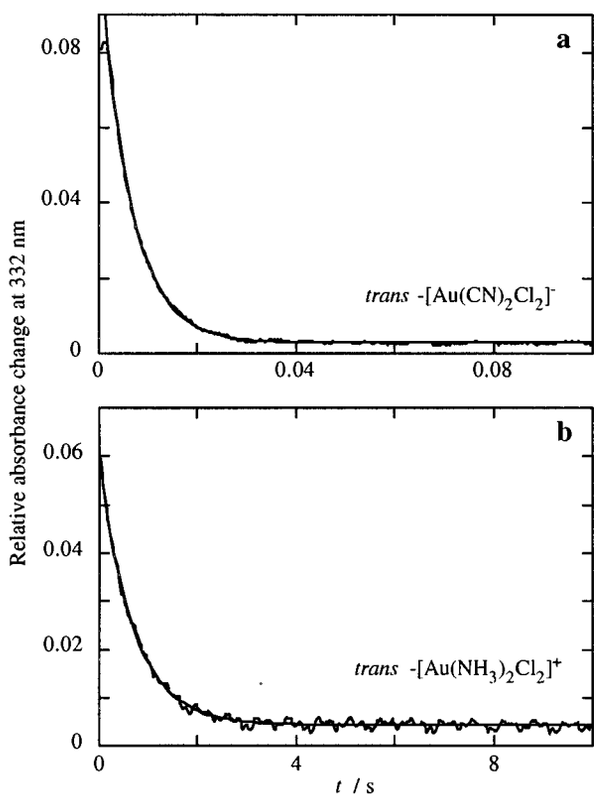


Fig. 1 Typical kinetic traces for the reaction of (a) d(^{s4}U) with *trans*-$[Au(CN)_2Cl_2]^-$ and (b) ^{s4}UMP with *trans*-$[Au(NH_3)_2Cl_2]^+$, at 25.0 °C and pH 4.0. The solid lines represent the least-squares fit of a single exponential function to the experimental data; $c_{Au} = (0.9–1.0) \times 10^{-4}$ mol dm^{-3}, [d(^{s4}U)], [^{s4}UMP] = 5.0×10^{-6} mol dm^{-3}, pH 4.0 buffered with $KHC_8H_4O_4$, $[Cl^-] = 5.0 \times 10^{-3}$ mol dm^{-3}, $I = 0.11$ mol dm^{-3}

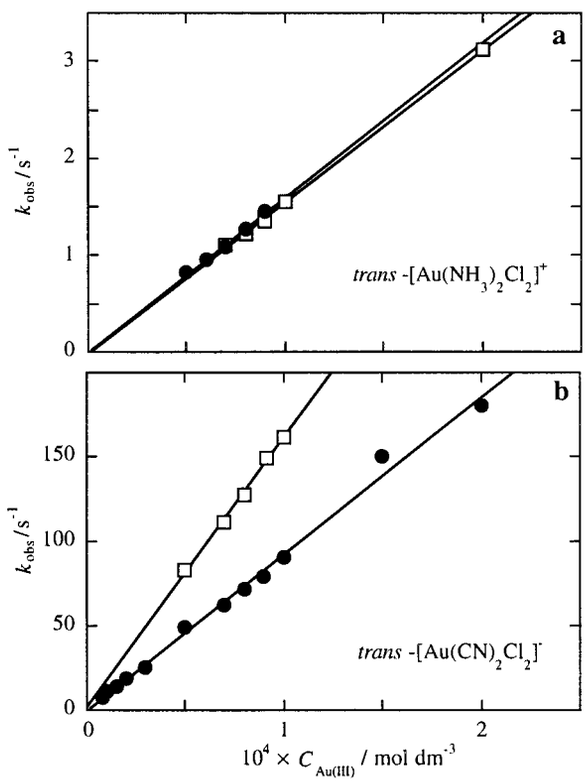


Fig. 2 Observed pseudo-first-order rate constants for the reaction of (□) d(^{s4}U) and (●) ^{s4}UMP with (a) *trans*-$[Au(NH_3)_2Cl_2]^+$ or (b) *trans*-$[Au(CN)_2Cl_2]^-$, as a function of total gold(III) concentration at 25.0 °C and pH 4.0; [d(^{s4}U)] = [^{s4}UMP] = (0.8–5.0) × 10^{-6} mol dm^{-3}, pH 4.0 buffered with $KHC_8H_4O_4$, $[Cl^-] = 5.0 \times 10^{-3}$ mol dm^{-3}, $\lambda = 332$ nm, $I = 0.11$ mol dm^{-3}

Throughout the whole pH range investigated, *trans*-$[Au(CN)_2Cl_2]^-$ reacts faster than *trans*-$[Au(NH_3)_2Cl_2]^+$. For example, at pH 4.0 with ^{s4}UMP, the second-order rate constants are 9.2×10^5 and 1.6×10^4 dm^3 mol^{-1} s^{-1}, respectively, *cf.* Table 1. This difference in reactivity can in part be attributed to the presence of cyanide ligands which contribute to stabilise the five-co-ordinated transition state in the substitution process due to better π-acceptor properties.[21] For reactions with the diammine complex, the second-order rate constants for the neutral d(^{s4}U) and the negatively charged ^{s4}UMP are almost identical, *cf.* Fig. 2(a). In contrast, the reaction of the dicyano complex with the negatively charged nucleophile ^{s4}UMP is significantly slower than the reaction with d(^{s4}U), *cf.* Fig. 2(b). In comparison with other sulfur donors the second-order rate constants for d(^{s4}U) and ^{s4}UMP are intermediate of those obtained for Me_2S and SCN^-,[36–38] suggesting a nucleophilicity trend according to Me_2S < d(^{s4}U), ^{s4}UMP < SCN^-, *cf.* Table 2.

pH and chloride ion dependence

In the four systems investigated an increase of pH results in a decrease of the second-order rate constants, *cf.* Table 1. Several factors might contribute to this variation, such as changes in the reactivity of the sulfur donor due to variations in the thione/thiol equilibrium or protonation/deprotonation equilibria of the phosphate group and/or heterocyclic nitrogens of the base as well as hydrolysis equilibria of the gold(III) complexes.[1,22,40,41,43,44] However, it is unlikely that pH-dependent equilibria involving the sulfur donor give rise to the observed

pH dependence, since spectroscopic and theoretical studies indicate that the thione form is the most stable and dominant species in the pH interval used.[22,44,45] Moreover, there is an increase in reactivity as the pH is decreased. This is contrary to what should be expected if the thiol is involved, since protonation usually results in formation of less reactive species.[46] Further, the monovalent phosphate group of ^{s4}UMP predominates in the pH range studied and the influence from protonation equilibria of the heterocyclic nitrogens is likely to be small.[1] Thus, distribution between chloro-, aqua- and hydroxo-gold(III) complexes with different reactivity seems to be a more plausible explanation for the pH dependence. However, in contrast to monoaqua complexes of Pd^{II} and Pt^{II}, those of Au^{III} are highly acidic,[21,47,48] *e.g.* pK_a $[AuCl_3(OH_2)] = 0.12$,[40] and their contribution to the reactivity should be small. The pH-dependent variation in reactivity should thus be due to a hydrolysis equilibrium of the type (3). An approximate equilibrium constant for reaction (3) can be estimated to be

$$trans\text{-}[AuY_2Cl_2]^{+/-} + H_2O \rightleftharpoons trans\text{-}[AuY_2Cl(OH)]^{+/-} + Cl^- + H^+ \quad (3)$$

$K \approx 10^{-7}$ mol dm^{-3} from available literature data.[40,41] Provided that the hydroxo complex is substantially less reactive than the dichloro complex, displacement of this equilibrium should result in a decrease of the observed rate constants around pH 5 ($[Cl^-] = 5 \times 10^{-3}$ mol dm^{-3}). This is in agreement with the observations for the reaction between ^{s4}UMP and *trans*-$[Au(CN)_2Cl_2]^-$ which was subject to a more detailed pH study as well as a variation of the chloride concentration at pH 5.0, see Table 1 (SUP 57364). A simplified expression for the apparent second-order rate constant can be derived according to equation (4) presupposing that *trans*-$[Au(CN)_2Cl_2]^-$ is the only species contributing to the observed reaction. A fit of equation (4) to the apparent second-order rate constants as a

$$k_{2,app} = \frac{k[Cl^-][H^+]}{K + [Cl^-][H^+]} \quad (4)$$

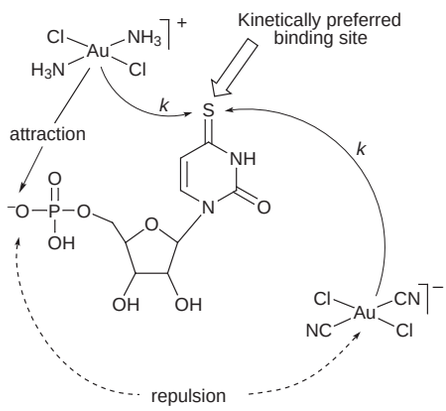
function of chloride concentration for the reaction of *trans*-$[Au(CN)_2Cl_2]^-$ with ^{s4}UMP at pH 5.0 resulted in a second-order rate constant $k = (10.7 \pm 1.0) \times 10^5$ dm^3 mol^{-1} s^{-1} for reaction (2) and an equilibrium constant for reaction (3) of $K = (1.2 \pm 0.2) \times 10^{-7}$ mol dm^{-3} (SUP 57364). The rate constant is in good agreement with that found experimentally at a pH where the dichloro complex predominates, $k = (10.4 \pm 0.9) \times 10^5$ dm^3 mol^{-1} s^{-1}, pH 2.9 and $[Cl^-] = 0.050$ mol dm^{-3}, see Table 1, and the equilibrium constant shows good agreement with the one estimated above. A fit of the kinetic traces according to the simple reaction model suggested above was also performed using the FACSIMILE computer program.[42] The resulting second-order rate constants given in Table 1 agree well in the pH range 2.9–5.0. At pH > 5.0, however, the observed second-order rate constants decrease more than expected from this model, possibly due to formation of hydroxo-bridged metal complexes and/or less reactive deprotonated species of the mono-nucleosides and -nucleotides.

Activation parameters

Temperature and pressure variations were made at pH 4.0 (SUP 57364), where the activation energy for the reaction described by the overall second-order rate constant should be dominated by the contribution from the substitution of chloride in the complex *trans*-$[AuY_2Cl_2]^{+/-}$ ($Y = CN^-$ or NH_3), as discussed above. All four reactions investigated exhibit negative entropies and volumes of activation together with relatively small enthalpies of activation. These observations suggest an associative interchange reaction mechanism, I_a, similar to that previously observed for other sulfur donors, *cf.* Table 2.[36–38,49,50]

Of the four reactions studied, the largest rate constant and smallest enthalpy of activation is obtained for the non-charged d(^{s4}U) with the softer complex *trans*-$[Au(CN)_2Cl_2]^-$. In this reaction the influence from electrostriction is minimised since the net charge of the transition state is the same as for the reactant metal complex. Thus, the negative $\Delta S^‡$ and $\Delta V^‡$ will mainly reflect the net decrease in entropy and volume in an associative activation process. The reaction of d(^{s4}U) with *trans*-$[Au(NH_3)_2Cl_2]^+$ is *ca.* 2 orders of magnitude slower than with *trans*-$[Au(CN)_2Cl_2]^-$, *cf.* Fig. 2. This decrease in reactivity is mainly an effect of an increased enthalpy of activation, which is expected in the absence of cyanide ligands stabilising the transition state. In addition, the reaction is further disfavoured by a more negative entropy of activation (−90 *vs.* −72 J K^{-1} mol^{-1}). This reaction also displays a substantially more negative volume of activation (−13 *vs.* −8.4 cm^3 mol^{-1}). Since the contribution from electrostriction is expected to be small also for the reaction of the positively charged complex with d(^{s4}U), this difference in $\Delta S^‡$ and $\Delta V^‡$ can be related to properties of the metal complex. One factor contributing to the discrepancy could for example be the better ability of the positively charged metal complex to form a structurally well organised hydration sphere which, if maintained in the transition state, would allow for a larger net decrease in volume and entropy during formation of the bond between the S-donor nucleophile and the metal centre.

In ^{s4}UMP a negative charge is introduced in the form of a phosphate group which also increases the steric bulk of the ligand compared to d(^{s4}U). At reaction with the negatively charged *trans*-$[Au(CN)_2Cl_2]^-$, both these changes contribute to decrease the apparent nucleophilicity of the ligand by (*i*) the repulsive charge interaction and (*ii*) the increased steric hinderance. As can be seen in Table 2 this reaction also has the largest enthalpy of activation. However, it is accompanied by a relatively small activation entropy (−40 J mol^{-1} K^{-1}) that contributes to maintain the reactivity at room temperature only slightly below that of d(^{s4}U) (−72 J K^{-1} mol^{-1}). A similar trend was also found for the activation volumes, −6.3 *vs.* −8.4 cm^3 mol^{-1} for ^{s4}UMP and d(^{s4}U), respectively. Thus, in contrast to many other reactions involving charge formation, the formation of a divalent negatively charged transition state does not seem to contribute to make $\Delta S^‡$ and $\Delta V^‡$ more negative compared to the reaction with the non-charged d(^{s4}U). One reason might be that the large distance between the charged phosphate and the S-donor does not allow a local build-up of net charge around the gold(III) centre where the chemical reaction takes place, *cf.* Scheme 1. In the case of reaction of ^{s4}UMP with the positively charged complex *trans*-$[Au(NH_3)_2Cl_2]^+$ the contributions from increased steric bulk and negative charge are expected to oppose each other. As mentioned above for



Scheme 1

Table 2 Rate constants and activation parameters at 25.0 °C for the reaction of polarisable ligands with gold(III) complexes

Reaction	k/dm^3 mol^{-1} s^{-1}	$\Delta H^{\ddagger}$/kJ mol^{-1}	$\Delta S^{\ddagger}$/J K^{-1} mol^{-1}	$\Delta V^{\ddagger}$/cm^3 mol^{-1}	Ref.
Me_2S + *trans*-$[Au(CN)_2Cl_2]^-$	$(2.0 \pm 0.1) \times 10^5$ [a] (4×10^5) [b]	25 ± 3	−54 ± 3	—	38
d(^{s4}U) + *trans*-$[Au(CN)_2Cl_2]^-$ [c]	$(1.6 \pm 0.1) \times 10^6$	16 ± 2	−72 ± 6	−8.4 ± 2.0	This work
d(^{s4}U) + *trans*-$[Au(NH_3)_2Cl_2]^+$ [c]	$(1.6 \pm 0.1) \times 10^4$	22 ± 3	−90 ± 9	−13.0 ± 1.0	This work
^{s4}UMP + *trans*-$[Au(CN)_2Cl_2]^-$ [c]	$(9.2 \pm 0.6) \times 10^5$	27 ± 3	−40 ± 4	−6.3 ± 1.2	This work
^{s4}UMP + *trans*-$[Au(NH_3)_2Cl_2]^+$ [c]	$(1.6 \pm 0.2) \times 10^4$	22 ± 3	−92 ± 9	−7.2 ± 0.7	This work
SCN^- + *trans*-$[Au(NH_3)_2Cl_2]^+$	$(3.3 \pm 0.2) \times 10^4$	33 ± 7	−48 ± 21	−4.5 ± 0.5	36,37
Br^- + *trans*-$[Au(NH_3)_2Cl_2]^+$	1075 ± 15	28 ± 1	−93 ± 4	—	50
SCN^- + $[AuCl_4]^-$	7.4	50	−59	—	49

[a] At 5.0 °C. [b] Rate constant at 25.0 °C calculated from the activation parameters. [c] pH = 4.0.

trans-$[Au(CN)_2Cl_2]^-$, the increased steric bulk should give a positive contribution to $\Delta H^{\ddagger}$. The charge interaction, on the other hand, should facilitate the rapid formation of an outer-sphere complex, and increase the probability of encounters with the metal centre. As can be seen from Table 2 these two effects seem to cancel; the $\Delta H^{\ddagger}$ values are 22 kJ mol^{-1} for both reactions. The introduction of the negative charge has no predictable influence on the entropy or volume of activation. The $\Delta S^{\ddagger}$ value is *ca.* −90 J K^{-1} mol^{-1} for both reactions, whereas the $\Delta V^{\ddagger}$ value for the reaction of ^{s4}UMP is significantly less negative compared to that obtained for d(^{s4}U), −7.2 *vs.* −13 cm^3 mol^{-1}. These effects may again be the result of the large distance between the metal centre and the phosphate group.

In summary, the activation parameters indicate that the quenching of the thione absorbance is the result of an associatively activated substitution process at the gold(III) centre. The reactions of positively charged metal complexes exhibit the most negative entropies of activation. This is possibly an effect of the better structured hydration sphere around the complex. The entropy term contributes to maintain the reactivity of *trans*-$[Au(NH_3)_2Cl_2]^+$ below that of *trans*-$[Au(CN)_2Cl_2]^-$, also when the electrostatic interactions favour the formation of the outer-sphere ion pair.

Conclusion

Rapidly reacting gold(III) complexes and their interactions with the thione unit of d(^{s4}U) and ^{s4}UMP can be used as model systems for metal ion–DNA interactions. The rate constants are mainly determined by the nature of the metal complex. The introduction of a single phosphate group, as a mimic for the neighbouring phosphodiester linkage in native DNA, has only a minor influence on the observed rate constants. A significant change in reactivity is found only when the electrostatic and steric factors co-operate, as observed for reaction with the negatively charged metal complex. By use of the cationic metal complex the favourable electrostatic interactions are sufficient only to maintain the reactivity of ^{s4}UMP similar to that of d(^{s4}U). The latter observation is in striking contrast to what has been observed for reactions of cationic metal complexes with short oligonucleotide fragments, where the contribution from electrostatic interactions increases the reactivity on the polymers dramatically compared to reference systems of mono- or di-nucleotides.[5,6,10,51]

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