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The substitution mechanism of $[Ru^{III}(edta)(H_2O)]^-$ with DNA bases, nucleoside and nucleotides in aqueous solution revisited †

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The substitution reactions of $[Ru^{III}(edta)(H_2O)]^-$ (edta = ethylenediaminetetraacetate) with adenine, adenosine and the corresponding 5′-nucleotides (Nu), *viz.* adenosine-5′-monophosphate (AMP), adenosine-5′-diphosphate (ADP) and adenosine-5′-triphosphate (ATP), have been studied kinetically as a function of nucleotide concentration at various temperatures (5 to 30 °C) at a fixed pH of 4.6 to contribute to the mechanistic understanding of the binding of adenine base nucleotides. Based on the kinetic results, it is suggested that the binding of the 5′-nucleotides (AMP, ADP and ATP) takes place in a rapid nucleophile concentration-dependent step, followed by a concentration-independent ring-closure reaction. Kinetic data and activation parameters have been interpreted in terms of an associative mechanism and discussed in reference to the data reported before.

Introduction

Coordination complexes of transition metals that bind in some selective way to DNA are of significant importance with respect to their use as chemotherapeutic agents or as tools in biotechnology for genetic engineering.[1–5] Ruthenium ammine and polyaminocarboxylate complexes have been proposed as alternatives to Pt(II) antitumor agents as reported in several reviews.[6–10] Recently, the $[Ru(H_2cydta)Cl_2]$ complex was shown to have antitumor activity towards Ehrlich ascitic tumors, P-388 leukemia cells and MX-1 transplanted carcinomas.[11] The lability of the coordinated water molecule in $[Ru^{III}(edta)(H_2O)]^-$ towards nucleophilic substitution offers the benefit of facile and straightforward synthesis of mixed-ligand complexes. In light of the above facts and considering that the adenine bases of DNA are potential binding sites for $[Ru(edta)(H_2O]^-$,[12] it seems essential to have kinetic and mechanistic information on the interaction of $[Ru(edta)(H_2O]^-$ with adenine base nucleotides in order to develop an understanding of Ru(III)–adenine base coordination in the presence of the phosphate-sugar unit in the nucleotides as a competitive coordination site.

Therefore, in the present study we have selected adenosine-5′-monophosphate (AMP), adenosine-5′-diphosphate (ADP) and adenosine-5′-triphosphate (ATP) as substituting nucleotides and studied the substitution of $[Ru^{III}(edta)(H_2O)]^-$ at pH 4.6 as a function of nucleotide concentration at various temperatures. Furthermore, while comparing the kinetic results reported earlier[12] for the substitution of the $[Ru^{III}(edta)(H_2O)]^-$ complex with adenine and adenosine, with those observed for the 5′-nucleotides (AMP, ADP and ATP), an apparent discrepancy came to our attention in that the 5′-nucleotides substitute the coordinated water molecule in $[Ru^{III}(edta)(H_2O)]^-$ much faster than reported for adenine and adenosine before.[12] We have, therefore, reinvestigated the kinetics of the water substitution in $[Ru^{III}(edta)(H_2O)]^-$ by adenine and adenosine, and have observed that both nucleophiles coordinate much faster to $[Ru^{III}(edta)(H_2O)]^-$ than found in an earlier study in which a hand-operated instead of an automated stopped-flow instrument was used.[12] In the present paper a detailed kinetic study and revision of our appraisal of the mechanism of binding of adenine, adenosine and the corresponding 5′-nucleotides are presented.

Experimental

$K[Ru^{III}(edta)Cl]\cdot 2H_2O$ was prepared and characterized according to a published procedure.[13] It rapidly aquates when dissolved in water and exists predominantly in its most labile aqua form, $[Ru^{III}(edta)(H_2O)]^-$, in the pH range 4–6.[14,15] All other chemicals used were of AR grade. Doubly distilled water was used throughout the experiments. Absorption spectra were recorded on a Cary 1G UV-Vis spectrophotometer equipped with a temperature controller. An SX 18 MV (Applied Photophysics) stopped-flow spectrophotometer coupled to an on-line data analysing system (PC) was employed in the kinetic measurements. The substitution reaction was followed at 330 nm where an appreciable spectral difference exists between reactant and product species. The instrument was thermostated to ±0.1 °C. Rate constant data were measured under pseudo-first order conditions in an excess (10–40 fold) of the nucleotides. pH measurements were carried out with a Mettler Delta 350 pH meter. Acetic acid–acetate buffer was used to maintain the pH of the kinetic solutions. Experimentally observed rate constants (k_{obs}) are presented as an average of several kinetic runs (at least five or six) and were found to be reproducible within 5%.

Results and discussion

Addition of nucleotides to an aqueous solution of $[Ru^{III}(edta)(H_2O)]^-$ resulted in the formation of $[Ru^{III}(edta)(Nu)]$ (Nu = AMP, ADP, ATP) as revealed by the spectral features[12] of the resulting solution. The change in absorbance in the UV region

† Electronic supplementary information (ESI) available: summary of pseudo-first order rate constants (s^{-1}) as a function of nucleophile concentration and temperature for the reaction of $[Ru^{III}(edta)(H_2O)]^-$ with AMP, ADP and ATP at pH 4.6. See http://www.rsc.org/suppdata/dt/b1/b108232a/

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(330–340 nm) was employed for kinetic measurements. When solutions of 5×10^{-4} M $[Ru^{III}(edta)(H_2O)]^-$ and nucleotides (0.005–0.02 M) were mixed in the stopped-flow apparatus at pH 4.6 using acetate buffer (the complex exists in its most labile form as $[Ru^{III}(edta)(H_2O)]^-$ in the pH range 4.0–6.0),[14,15] two subsequent exponential traces (both accompanied by an increase in absorbance at 330 nm) were observed, as shown in Fig. 1 for the case of AMP as an example for the 5′-nucleotides

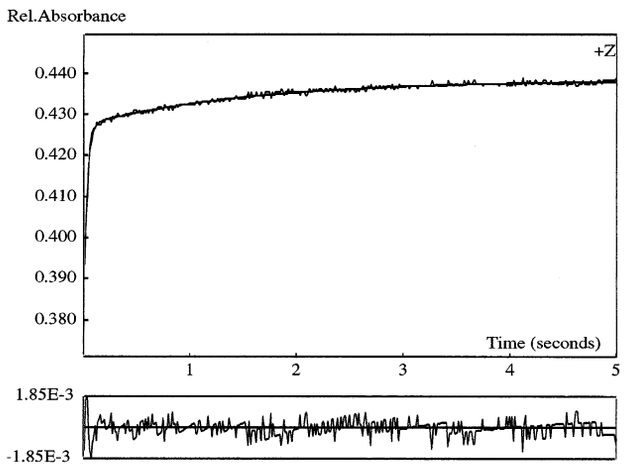


Fig. 1 Typical kinetic trace for the reaction between 5×10^{-4} M $[Ru(edta)(H_2O)]^-$ and 0.005 M AMP, pH 4.6 (acetate buffer) and 25 °C. The trace was fitted to two exponentials by following the increase in absorbance at 330 nm. The lower trace represents the difference between the experimental and calculated curves.

used in this study. The observed rate constant (k_{obs}) for the fast step increased linearly with increasing [Nu] with an appreciable intercept, signifying the operation of a reverse aquation reaction of the substituted product. The plots in Figs. 2a–c report the effect of [AMP], [ADP] and [ATP] on the reaction of $[Ru^{III}(edta)(H_2O)]^-$ with these ligands, respectively, as a function of temperature (5–30 °C). The slopes and intercepts of these plots give the values of k_1 and k_{-1}, respectively, which are listed in Table 1. The rate law for this fast step is given by eqn. (1).

$$k_{obs} = k_1[Nu] + k_{-1} \quad (1)$$

The rate constants corresponding to the slower step were found to be independent of the 5′-nucleotide concentrations. The values of the rate constants and the corresponding thermal activation parameters are summarised in Table 1. A comparison of the rate data obtained in the present study for the reaction with 5′-nucleotides with those reported before for the reaction with adenine and adenosine[12] reveals that the 5′-nucleotides react much faster with $[Ru^{III}(edta)(H_2O)]^-$ than found for adenine and adenosine. This trend appears to be quite unusual since the incorporation of a phosphate-sugar group onto the adenine base is not expected to add much to the nucleophilicity of the adenine base.

This prompted us to reinvestigate the kinetics of the reaction of 5×10^{-4} M $[Ru^{III}(edta)(H_2O)]^-$ with adenine and adenosine (0.005–0.02 M). It was found that adenine and adenosine react with $[Ru^{III}(edta)(H_2O)]^-$ in a similar manner, but at a faster rate ($k_1 = 8840 \pm 501$ M^{-1} s^{-1}, $k_{-1} = 15 \pm 7$ s^{-1} for adenine, and $k_1 = 8902 \pm 379$ M^{-1} s^{-1} and $k_{-1} = 13 \pm 5$ s^{-1} for adenosine at 25 °C and pH 4.6) than the 5′-nucleotides (see Table 1). The values of the second order rate constant (k_1) reported before, *viz.* 400 ± 10 and 110 ± 4 M^{-1} s^{-1} for adenine and adenosine, respectively,[12] are indeed for the slower step of the overall reaction. In fact, this step should have been independent of concentration of adenine or adenosine as observed in the present study. The reason for the observed concentration dependence for the slower step,[12] is probably associated with the inability to detect

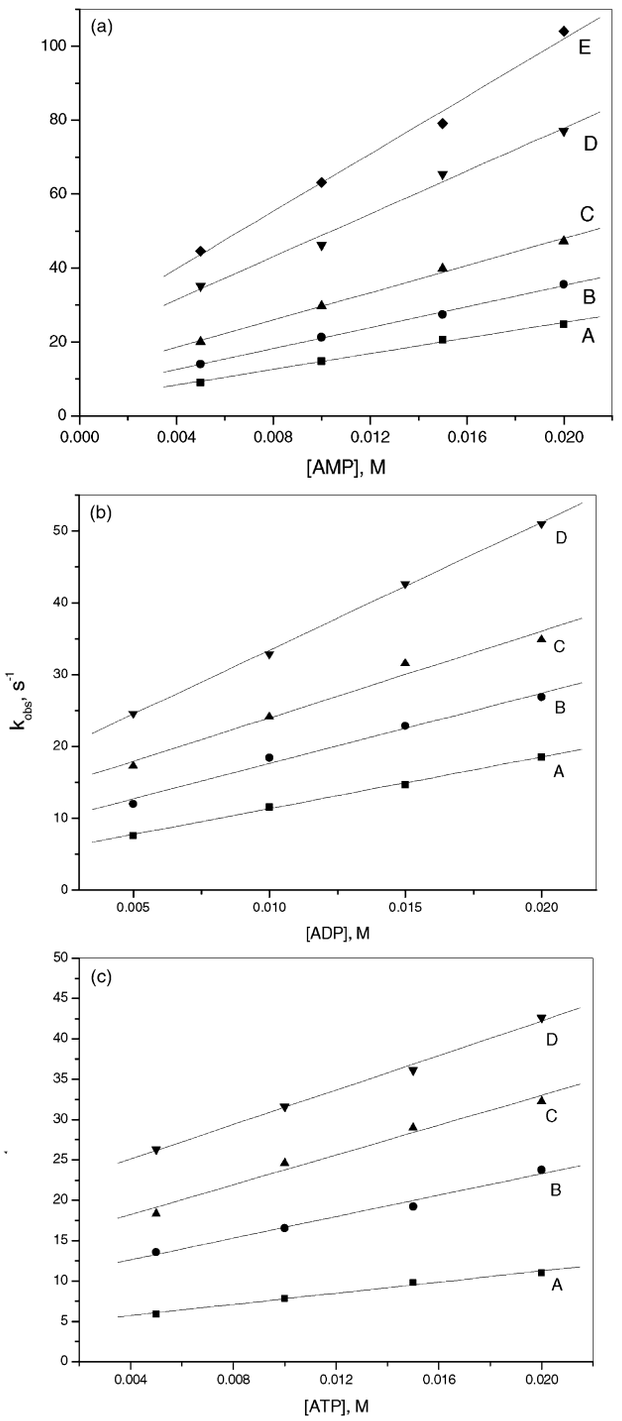


Fig. 2 (a) Plot of k_{obs} *versus* [AMP] for the reaction between $[Ru(edta)(H_2O)]^-$ and AMP as a function of temperature. Experimental conditions: $[Ru(edta)(H_2O)]^- = 5 \times 10^{-4}$ M, pH 4.6, T = 5.0 (A), 10.0 (B), 15.0 (C), 25.0 (D) and 30.0 °C (E). (b) Plot of k_{obs} *versus* [ADP] for the reaction between $[Ru(edta)(H_2O)]^-$ and ADP as a function of temperature. Experimental conditions: $[Ru(edta)(H_2O)]^- = 5 \times 10^{-4}$ M, pH 4.6, T = 5.0 (A), 10.0 (B), 15.0 (C) and 20.0 (D). (c) Plot of k_{obs} *versus* [ATP] for the reaction between $[Ru(edta)(H_2O)]^-$ and ATP as a function of temperature. Experimental conditions: $[Ru(edta)(H_2O)]^- = 5 \times 10^{-4}$ M, pH 4.6, T = 5.0 (A), 15.0 (B), 20.0 (C) and 25.0 °C (D).

the faster kinetic step clearly with the available instrumentation, such that the slower step includes a residual contribution of the faster step and causes an increase in the observed rate constants with increasing concentration of adenine and adenosine.

In view of the general kinetic behaviour observed in the present study for the reactions of $[Ru^{III}(edta)(H_2O)]^-$ with adenine, adenosine, AMP, ADP and ATP, and considering the

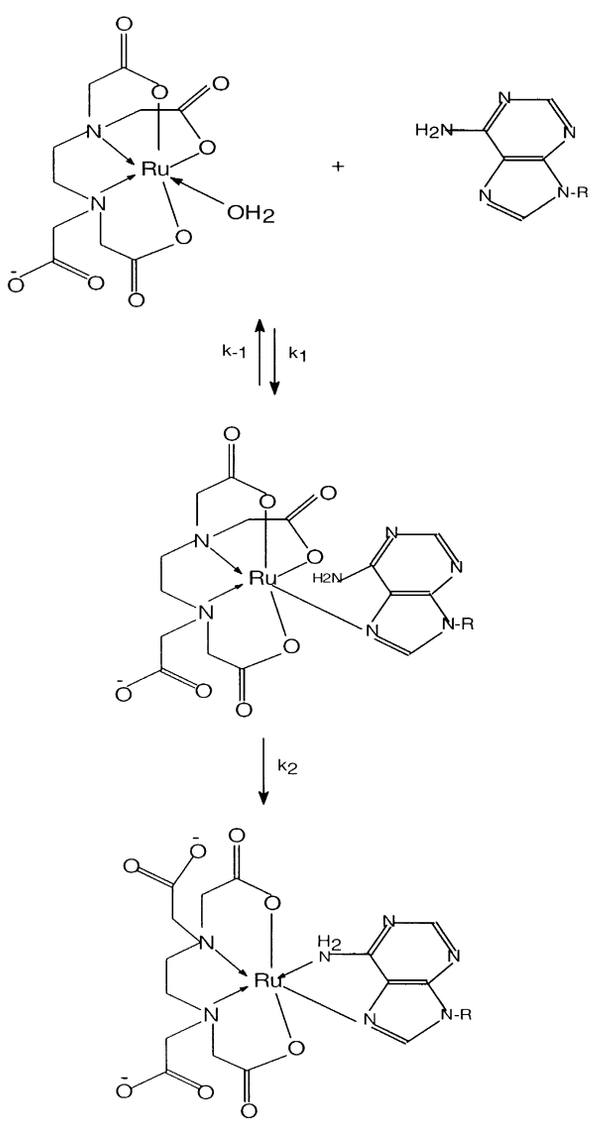
Table 1 Rate and activation parameters for the reaction of $[Ru^{III}(edta)(H_2O)]^-$ with adenine, adenosine and adenosine-5′-phosphates[a]

Nu	T/°C	k_1/M^{-1} s^{-1}	k_{-1}/s^{-1}	k_2/s^{-1}
AMP	5	1058 ± 53	4.1 ± 0.7	
	10	1418 ± 55	6.9 ± 0.8	
	15	1852 ± 81	11.1 ± 1.1	
	25	2904 ± 221	19.8 ± 3	0.68 ± 0.03
	30	3888 ± 260	24.2 ± 3.6	
		$\Delta H_1^{\neq}$ = 33 ± 1 kJ mol^{-1}	$\Delta H_{-1}^{\neq}$ = 47 ± 4 kJ mol^{-1}	
		$\Delta S_1^{\neq}$ = −67 ± 3 J K^{-1} mol^{-1}	$\Delta S_{-1}^{\neq}$ = −63 ± 15 J K^{-1} mol^{-1}	
ADP	5	720 ± 23	4.1 ± 0.3	
	10	982 ± 80	7.8 ± 1.0	
	15	1206 ± 130	11.9 ± 1.8	
	20	1786 ± 41	15.5 ± 0.6	0.65 ± 0.05
		$\Delta H_1^{\neq}$ = 37 ± 4 kJ mol^{-1}	$\Delta H_{-1}^{\neq}$ = 58 ± 8 kJ mol^{-1}	
		$\Delta S_1^{\neq}$ = −55 ± 12 J K^{-1} mol^{-1}	$\Delta S_{-1}^{\neq}$ = −25 ± 26 J K^{-1} mol^{-1}	
ATP	5	345 ± 24	4.3 ± 0.3	
	15	672 ± 59	9.9 ± 0.8	
	20	928 ± 96	14.5 ± 1.3	
	25	1068 ± 55	20.8 ± 0.8	0.62 ± 0.04
		$\Delta H_1^{\neq}$ = 38 ± 4 kJ mol^{-1}	$\Delta H_{-1}^{\neq}$ = 52 ± 0.5 kJ mol^{-1}	
		$\Delta S_1^{\neq}$ = −59 ± 12 J K^{-1} mol^{-1}	$\Delta S_{-1}^{\neq}$ = −45 ± 2 J K^{-1} mol^{-1}	
Adenine	25	8840 ± 501	15 ± 7	4.4 ± 0.1
Adenosine	25	8902 ± 379	13 ± 5	1.06 ± 0.04

[a] Observed rate constants are summarized in the ESI,† [Ru(III)] = 5×10^{-4} M, pH = 4.6.

earlier reports[16] on the kinetics of chelate formation with the $[Ru^{III}(edta)(H_2O)]^-$ complex, the present kinetic results may be interpreted in terms of a rapid formation of a mono-ligated product (through N-7 of the adenine base), followed by a ring-closure step (independent of the nucleophile concentration) in which the exocyclic NH_2 group (at C-6 of the adenine base) is coordinated to the ruthenium center by diplacement of a coordinated carboxylate group of edta (Scheme 1). The N-7 binding mode of adenine and guanine bases with ruthenium complexes as well as with other transition metal complexes has been well documented in the literature.[17] A recent report[18] on spectral studies of the $[Ru^{III}(edta)(H_2O)]^-$ complex with 5′-GMP further supports that N-7 is a potential binding site in purine bases. Binding through the phosphate moiety of the 5′-nucleotides has been ruled out since the reaction of $[Ru^{III}(edta)(H_2O)]^-$ with phosphate is very slow.

The activation parameters for the reaction of $[Ru^{III}(edta)(H_2O)]^-$ with AMP, ADP and ATP are summarized in Table 1. The values of $\Delta H^{\neq}$ and $\Delta S^{\neq}$ found for the reaction with AMP are 33 ± 1 kJ mol^{-1} and −67 ± 3 J K^{-1} mol^{-1}, respectively. However, in the case of ADP and ATP, values of $\Delta H^{\neq}$ and $\Delta S^{\neq}$ were found to be 37 ± 4 kJ mol^{-1} and −55 ± 12 J K^{-1} mol^{-1}, and 38 ± 4 kJ mol^{-1} and −59 ± 12 J K^{-1} mol^{-1}, respectively. The low values of $\Delta H^{\neq}$ and negative values of $\Delta S^{\neq}$ for the substitution reactions of $[Ru^{III}(edta)(H_2O)]^-$ clearly support the associative character of the substitution process. These values are in a good agreement with those reported in the literature for related reactions of $[Ru^{III}(edta)(H_2O)]^-$ with a series of neutral and anionic ligands.[14,15] Significantly negative entropies of activation ($\Delta S^{\neq}$) and negative volumes of activation ($\Delta V^{\neq}$) were reported for the reactions of thiourea, dimethylthiourea, tetramethylthiourea, thiocyanate and azide with ethylenediaminetetraacetate and related complexes of Ru(III) (hedta, hedtra, medtra),[15,19,20] and were found to be in the range of −75 to −139 J K^{-1} mol^{-1} and −4.1 to −12.2 cm^3 mol^{-1}, respectively. These values strongly support the associative mechanism for the substitution reactions of $[Ru^{III}(edta)(H_2O)]^-$.



R = H for DNA bases
R = Deoxyribose for nucleosides
R = 5′ -Deoxyribophosphate for nucleotides

Scheme 1

Conclusions

The present investigation provides new mechanistic information on the reaction of $[Ru^{III}(edta)(H_2O)]^-$ with the adenine base and the corresponding 5′-nucleotides. The reactivity order AMP > ADP > ATP is in agreement with the size of the nucleotides. Formation of the chelated product with 5′-nucleotides

in comparison with *cis*-platin indicates the possible future prospect of the Ru(edta) type of complexes with respect to their probable oncological application in cancer treatment.

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