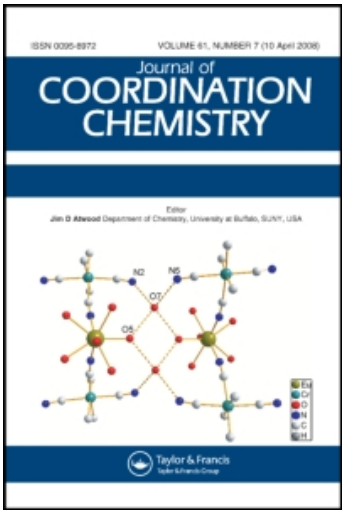

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Kinetics and mechanism of the reaction of $[Ru^{II}(tpy)(pic)(H_2O)]^+$ with $KHSO_5$ in oxidative cleavage of DNA

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Reaction of $[Ru^{II}(tpy)(pic)(H_2O)]^+$ (**1**) with $KHSO_5$ resulting in the formation of $[Ru^{IV}(tpy)(pic)(O)]^+$ (**2**) was studied kinetically as a function of $[KHSO_5]$, temperature (15–35°C), and pressure (10–30 MPa) at a fixed pH of 5.1 using spectrophotometric techniques. A suggested mechanism that is in agreement with the observed rate and activation parameters is presented. Complex **1** was found to induce DNA (pBluescript) cleavage in the presence of $KHSO_5$, which proceeds *via* oxygen transfer from **2**.

Keywords: Ruthenium; Oxone; Kinetics; DNA cleavage

1. Introduction

Although the kinetics and mechanism of oxo-transfer reactions of Ru(IV)-oxo complexes containing polypyridyl ligands, such as $[Ru(bipy)_2(py)(O)]^{2+}$ (bipy = 2,2′-bipyridine) [1–7] and $[Ru^{IV}(tpy)(bipy)(O)]^{2+}$ (tpy = 2,2′,6′,2″-terpyridine) [8–12], have been studied in detail, reports on the formation kinetics and mechanism of such Ru(IV)-oxo complexes in the reaction of precursor Ru(II) complexes with oxygen atom transfer agents are scarce. In recent studies [13–15] on the synthesis and catalytic ability of a new $[Ru^{II}(tpy)(pic)(H_2O)]^+$ ($pic^- =$ picolinate) complex (**1**) toward epoxidation of various alkenes in the presence of *t*-BuOOH as a terminal oxidant, we reported results of a brief kinetic study of reaction of **1** with *t*-BuOOH [13]. In this study, we have selected $KHSO_5$ as precursor oxidant, since $KHSO_5$ is a reasonably strong oxidant ($E^0 = 1.82$ V) [16] which is readily available and stable in the solid state as a "triple salt" ($2KHSO_5$, K_2SO_4, $KHSO_4$). In the pH range 6.0–8.0, it exists in aqueous solution as HSO_5^-, whereas at higher pH (> 10.5), it exists as SO_5^{2-}. Reports on the use of $KHSO_5$ in DNA cleavage in the presence of water–soluble transition metal complexes are available in the

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literature [17, 18]. In this article, we report kinetic and mechanistic studies on the reaction of **1** with $KHSO_5$ and the ability of **1** to cleave DNA in the presence of $KHSO_5$ as oxidant.

2. Experimental

2.1. *Materials*

$[Ru^{II}(trpy)(pic)H_2O]ClO_4$ (**1**) was prepared by following the procedure reported earlier [13] and characterized by comparing the spectral (UV-Vis and IR) and micro-analysis data. All chemicals used were of reagent grade, obtained from Aldrich Chemical Company, and appropriately degassed before use. Doubly distilled water was used in these studies.

2.2. *Instrumentation*

The UV-Vis and IR spectral data for **1** were collected on Perkin Elmer Model Lambda 35 and Model 783 spectrophotometers using KBr pellets, respectively. A Perkin Elmer 240C elemental analyzer was used to obtain micro-analytical (C, H, and N) data for **1**.

2.3. *Kinetic studies*

The kinetics of the reaction of **1** with $KHSO_5$ was studied spectrophotometrically using a Perkin Elmer (Model Lambda 35) spectrophotometer. The solution temperature was maintained to within $\pm 0.1^{\circ}C$ using a circulating water bath (Julabo MP-5). The rate of the reaction was followed by monitoring the decrease in absorbance of **1** at 500 nm in water under pseudo-first-order conditions of excess $KHSO_5$ (10–40 times) at a constant pH of 5.1 using 0.2 mol L^{-1} acetate buffer. High-pressure kinetic measurements were performed on a homemade high-pressure stopped-flow instrument [19] at pressures up to 130 MPa. Experimentally observed rate constants (k_{obs}) are reported as the average of at least five to six kinetic runs and are reproducible within $\pm 5\%$.

2.4. *DNA cleavage study*

Oxidative DNA damage ensuing from the reaction between **1** and $KHSO_5$ was determined by plasmid relaxation assay as described earlier [20]. Briefly, plasmid pBluescript SK^+ DNA was isolated using QIAGEN Plasmid Mini Kit and reactions performed in 200 μL PCR tubes using 1 μg of plasmid DNA, $KHSO_5$ (0–4 mmol L^{-1}) and/or **1** (250 μmol L^{-1}) in 20 mmol L^{-1} phosphate buffer (final volume 25 μL). All reactants excluding plasmid DNA were pre-incubated at 37°C for 1 h to ensure complete formation of the Ru(IV)-oxo species under the selected conditions. Plasmid DNA was introduced subsequently and further incubated for 1 h at 37°C. Samples were then loaded on an agarose gel (1.2%) containing ethidium bromide (0.5 μg mL^{-1}) and subjected to electrophoresis for 90 min. The supercoiled (Form I) and open circular (Form II) forms of plasmid were quantified by densitometric analysis for each

treatment using TotalLab Nonlinear Dynamic Image analysis software (Nonlinear USA Inc., Durham, USA). The conversion from Form I into Form II was represented as percentage of plasmid relaxation.

3. Results and discussion

Micro-analysis and spectral (UV-Vis and IR) data of **1** were found to be very close to the data reported previously [13]. In the absorption spectrum of **1**, the strong band appearing in the visible region (figure 1) is attributed to metal-to-ligand, $d\pi(Ru) \rightarrow \pi^*$(polypyridyl) charge transfer transition. Spectral changes that occurred upon addition of $KHSO_5$ to an aqueous solution of **1** are shown in figure 1, whereas the inset of figure 1 shows the corresponding absorbance–time trace at 500 nm. Preliminary experiments established that the reaction of **1** with buffer components (acetate or phosphate) is negligibly slow compared to that with $KHSO_5$ under similar experimental conditions. Under the specified conditions, the rate of the reaction was found to be first order with respect to **1**. The effect of $[KHSO_5]$ on the values of the pseudo-first-order rate constant (k_{obs}) is shown in figure 2 as a function of temperature. The plots in figure 2 are slightly curved and suggest a mechanism that consists of a rapid pre-equilibrium followed by a rate-determining oxidation step as shown in reactions (1) and (2):

$$\underset{(\mathbf{1})}{\left[Ru^{II}(tpy)(pic)(H_2O)\right]^+} + HSO_5^- \underset{}{\overset{K}{\rightleftharpoons}} \left[Ru^{II}(tpy)(pic)(OOSO_3)\right]^- + H_3O^+ \qquad (1)$$

$$\left[Ru^{II}(tpy)(pic)(OOSO_3)\right]^- \xrightarrow{k} \underset{(\mathbf{2})}{\left[Ru^{IV}(tpy)(pic)O\right]^+} + SO_4^{2-} \qquad (2)$$

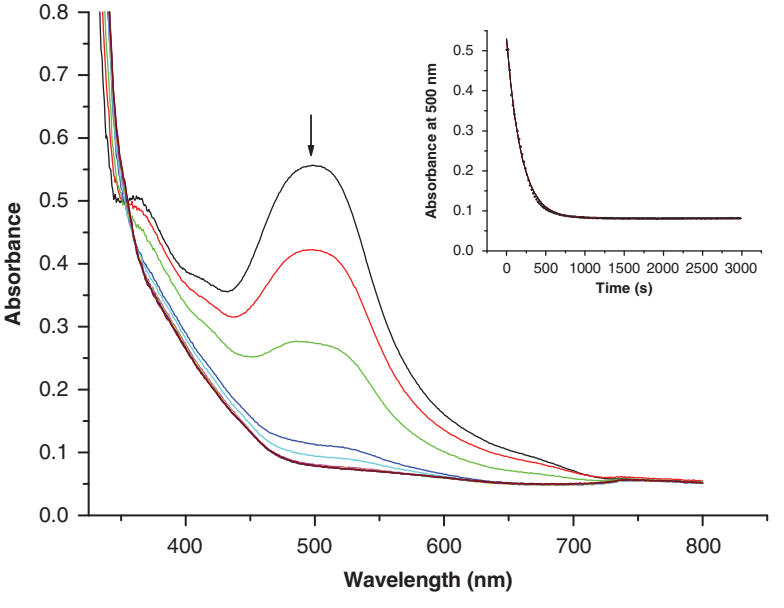

Figure 1. Spectral changes that occur during the reaction of **1** (1.5×10^{-4} mol L^{-1}) with $KHSO_5$ (1.5×10^{-3} mol L^{-1}) in water at 25°C. Inset: kinetic trace at 500 nm.

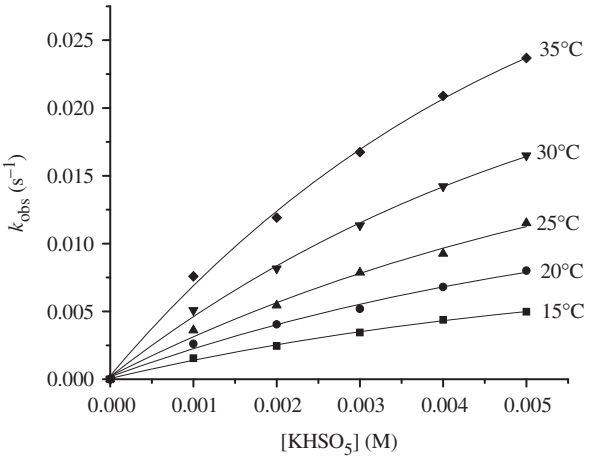

Figure 2. Plot of k_{obs} *vs.* [$KHSO_5$] at different temperatures. [**1**] = 1.5×10^{-4} mol L^{-1}, pH = 5.1 (0.2 mol L^{-1} acetate buffer).

for which the following rate law can be derived.

$$k_{obs} = kK[HSO_5^-]/(1 + K[HSO_5^-]) \quad (3)$$

or

$$1/k_{obs} = 1/k + 1/kK[HSO_5^-]. \quad (4)$$

The suggested mechanism consists of rapid ligand substitution during which coordinated water is reversibly displaced by HSO_5^-, which on coordination deprotonates to SO_5^{2-}, peroxomonosulfate. In the subsequent slow reaction, heterolytic cleavage of the peroxo bond leads to the formation of the Ru(IV)-oxo complex and sulfate. The Ru(IV)-oxo complex (**2**) so produced is capable of cleaving DNA as reported below.

The values for k and K determined from the intercept and slope of the plots of $1/k_{obs}$ *versus* $1/[HSO_5^-]$ (figure 3) at different temperatures are given in table 1. The data show that K is relatively small, which accounts for the weak curvature observed in the plots in figure 2, and is independent of temperature within the error limits of the data. The values of k at different temperatures were used to construct the Eyring plot (figure 4) from which $\Delta H^{\neq}$ and $\Delta S^{\neq}$ were determined (table 1). The values of the activation parameters, particularly the negative values of $\Delta S^{\neq}$, are consistent with reaction (2) that involves oxidation of the metal center (Ru^{II}–Ru^{IV}) that is accompanied by overall charge creation and an increase in electrostriction. Both these contributions will lead to a decrease in entropy.

The effect of pressure on the overall reaction was studied at 25°C ([$KHSO_5$] = 0.005 mol L^{-1}), for which the data are shown in figure 5. The reaction is significantly accelerated by pressure and accompanied by a negative volume of activation of -16.8 ± 0.4 cm^3 mol^{-1}. Under the selected conditions, it is reasonable to assume that the observed pressure dependence is for the overall second-order rate constant kK as a result of the weak curvature in the plots of k_{obs} *versus* $[HSO_5^-]$ (figure 2).

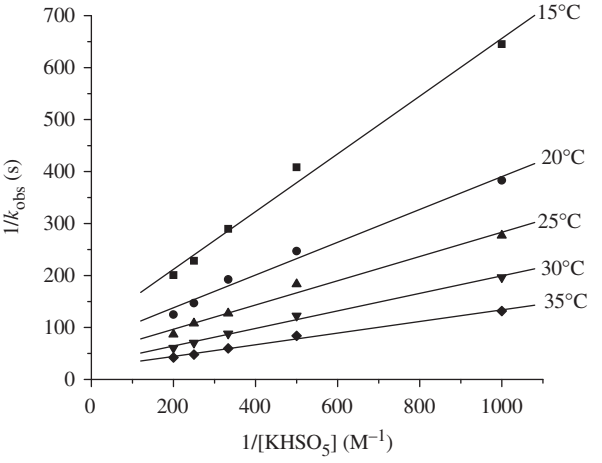

Figure 3. Plot of $1/k_{obs}$ *vs.* $1/[KHSO_5]$ at different temperatures. $[\mathbf{1}] = 1.5 \times 10^{-4}\,mol\,L^{-1}$, pH = 5.1 ($0.2\,mol\,L^{-1}$ acetate buffer).

Table 1. Rate and activation parameters for the reaction of **1** with $KHSO_5$.

Temperature (°C)	$k \times 10^2$ (s^{-1})	K ($mol\,L^{-1}$)$^{-1}$	$\Delta H^{\neq}$ ($kJ\,mol^{-1}$)	$\Delta S^{\neq}$ ($J\,K^{-1}\,mol^{-1}$)
15	1.0 ± 0.2	180 ± 32	55 ± 3	-92 ± 9
20	1.3 ± 0.2	234 ± 40		
25	2.0 ± 0.4	217 ± 47		
30	3.3 ± 0.5	179 ± 28		
35	4.5 ± 0.7	199 ± 33		

$[\mathbf{1}] = 1 \times 10^{-4}\,mol\,L^{-1}$, pH = 5.1 ($0.2\,mol\,L^{-1}$ acetate buffer).

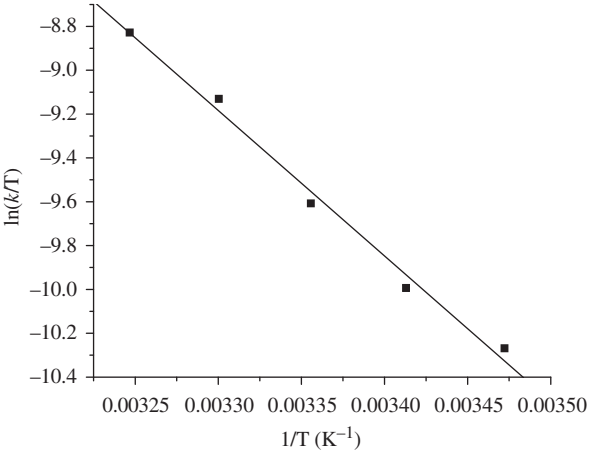

Figure 4. Eyring plot of $\ln(k/T)$ *vs.* $1/T$.

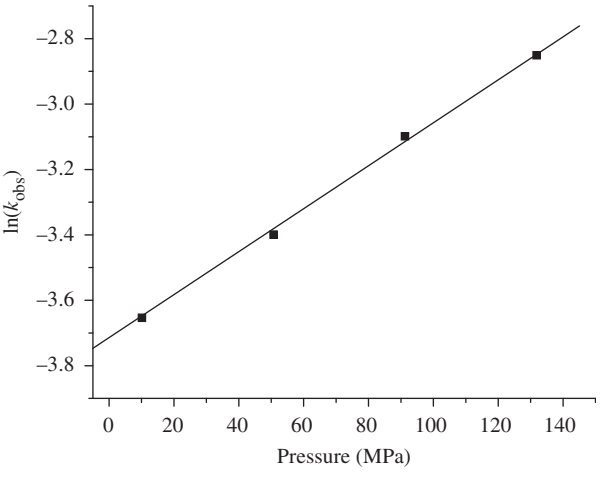

Figure 5. Plot of $\ln k_{obs}$ *vs.* pressure for the reaction of **1** with $KHSO_5$ at 25°C. $[\mathbf{1}] = 1.0 \times 10^{-4}\,mol\,L^{-1}$, $[KHSO_5] = 5.0 \times 10^{-3}\,mol\,L^{-1}$, pH = 5.1 (0.2 mol L^{-1} acetate buffer).

Furthermore, since reaction (1) consists of a ligand substitution reaction with no overall change in charge, it is reasonable to predict that K will show no significant pressure dependence. The observed negative volume of activation must then be assigned to the pressure dependence of reaction (2), which can again be accounted for in terms of the oxidation of the metal center and significant charge creation which will be accompanied by an increase in electrostriction, and both contributions will be accompanied by a volume collapse.

The potential of **1** to cleave DNA in the presence of the primary oxidant $KHSO_5$ was studied by gel electrophoresis using supercoiled pBluescript (SK^+) plasmid DNA (SC) in Tris buffer (pH 7.8). DNA cleavage efficiency of **1** was monitored by observing the conversion of supercoiled (Form I) plasmid DNA to the circular nicked form (Form II). Shown in figure 6 are the results of cleavage of supercoiled plasmid DNA. The gel shows the inability of **1** alone to bring on any apparent cleavage of DNA (figure 6, lane 2). Results of our control experiments also revealed that in the absence of the Ru^{III}–EDTA complex the primary oxidant $KHSO_5$ does not cause any significant cleavage of DNA (figure 6, lanes 3–5) under the specified conditions. However, **1** shows an appreciable conversion of supercoiled pBluescript (SK^+) DNA (Form I) to nicked circular (Form II) DNA upon prolonged incubation in the presence of $KHSO_5$. Efficacy of DNA cleavage by **1** increased with increasing amount of added $KHSO_5$ (figure 6, lanes 6–8). The DNA cleavage ability of high-valent ruthenium-oxo complexes containing polypyridyl ligands is well-documented [21–25]. Reaction of **1** with $KHSO_5$ leads to the formation of high-valent Ru(IV)-oxo species which is capable of the C–H bond activation in phenol oxidation [14]. We presume that the high-valent $[Ru^{IV}(tpy)(pic)O]^+$ (**2**) formed in the reaction of **1** and $KHSO_5$ accomplishes DNA cleavage similar to $[Ru^{IV}(tpy)(bipy)O]^{2+}$ (bipy = 2,2′-bipyridyl) [21–23] and other related Ru(IV)-oxo complexes [9, 10]. Based on the kinetic and product analysis data reported earlier for the oxidation of nucleotides (constituents of DNA) by Ru(IV)-oxo complexes [22], it was suggested that AMP (adenosine-5′-monophosphate), CMP (cytidine-5′-monophosphate), and TMP (thymidine-5′-monophosphate) undergo

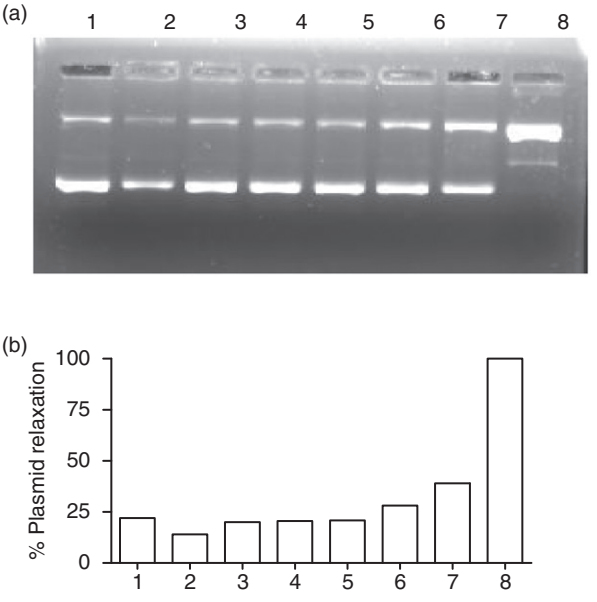

Figure 6. (a) Cleavage of pBluescript (SK^+) plasmid DNA by **1** in the presence of $KHSO_5$. DNA was treated (see Sections 2 and 3) and subsequently electrophoresed on agarose gel (1.2%), followed by densitometric analysis. Lane 1: Control DNA, lane 2: DNA incubated with **1** (250 μmol L^{-1}), lanes 3–5: DNA incubated with 2, 3, and 4 mmol L^{-1} of $KHSO_5$, respectively, and lanes 6–8: DNA co-incubated with 250 μmol L^{-1} of **1** and 2, 3, and 4 mmol L^{-1} of $KHSO_5$, respectively. (b) Percentage of plasmid relaxation.

oxidation at their corresponding sugar moiety, whereas oxidation of GMP (guanosine-5′-monophosphate) by $[Ru^{IV}(tpy)(bipy)O]^{2+}$ takes place through its base (guanine) unit [23]. Oxidation of the sugar moiety of nucleotides involves abstraction of a hydrogen atom from deoxyribose leading to sugar fragmentation followed by base release and DNA cleavage [22]. Other oxo-metal species oxidize DNA both at 1′ and 5′ sites of the sugar unit [26, 27]. The guanine oxidation by $[Ru^{IV}(tpy)(bipy)O]^{2+}$ involved an inner-sphere electron transfer pathway [23]. The reactivity of $[Ru^{IV}(tpy)(bipy)O]^{2+}$ toward nucleotide oxidation is as follows GMP $\gg$ AMP > CMP > TMP [22, 23]. We propose that $[Ru^{IV}(tpy)(pic)O]^{+}$ formed in the reaction of **1** with $KHSO_5$ cleaves DNA by following parallel guanine and sugar oxidation pathways as exhibited by $[Ru^{IV}(tpy)(bipy)O]^{2+}$ [23]. We did not observe any DNA cleavage when $KHSO_5$ was substituted by H_2O_2 (results of gel electrophoresis are provided in the on-line Supplementary material). We also found that reaction of **1** with H_2O_2 was very slow, and did not lead to the formation of oxoruthenium(IV) which is believed to be responsible for DNA cleavage [21–23].

4. Conclusion

Although ruthenium(IV)-oxo-polypyridyl complexes are known for stoichiometric oxidation of DNA, reports on the catalytic application of their ruthenium(II)-analogs in oxidation of DNA are absent (albeit one report on the electrocatalytic DNA cleavage

exists in the literature [21]). Thus, the results of this study demonstrates for the first time that **1** acts as an efficient catalyst for the cleavage of supercoiled plasmid DNA by a precursor oxidant $KHSO_5$ through the formation of an active ruthenium(IV)-oxo complex **2**.

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