Kinetics and mechanism for the substitution reactions of monoaquamonochloro-(piperazine) palladium (II) complex with L-methionine and thiourea in aqueous solution

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Abstract. The interaction of the palladium(II) complex $[\text{Pd}(\text{Pip})\text{Cl}(\text{H}_2\text{O})]^+$, where Pip is piperazine, with the sulphur donor nucleophiles; L-methionine (L-Met), and thiourea (tu) was studied under pseudo-first-order conditions as a function of nucleophile concentration and temperature, using UV-Visible spectrophotometric and stopped-flow techniques. Two consecutive reaction steps were observed for the substitution process with L-methionine. For the substitution with thiourea, a third reaction step, the displacement of the labilized amine, as a result of the strong *trans*-effect of thiourea ligand was observed. ^1H NMR spectroscopy was used to follow the substitution of the ligand by thiourea. The activation parameters for all reactions studied suggest an associative substitution mechanism.

Keywords. Palladium (II); piperazine; sulphur donor nucleophiles; stopped-flow spectrophotometry; activation parameters.

1. Introduction

Despite the clinical anticancer utility of cisplatin, carboplatin, oxaliplatin, and several other complexes in clinical trials, there is a continued interest in the design of new complexes that shows antitumour activities equivalent or better than these agents.^{1–5} The continued interest in platinum-based antitumour compounds is stimulated by the fact that certain tumours are resistant to the clinically used drugs cisplatin and carboplatin. The similarity between the coordination chemistry of platinum (II) and palladium (II) compounds supports the theory that palladium complexes can act successfully as antitumour drugs and show fewer side effects relative to other heavy metal anticancer compounds.⁶ Pd(II) analogues of Pt(II) complexes are well-suited for kinetic and mechanistic studies by application of rapid-mixing techniques. Recently interactions of Pt(II) anticancer drugs and their Pd(II) analogues with sulphur containing amino acid side chains have attracted much attention in studies on the biological activity of cisplatin^{7–9} and carboplatin.¹⁰ These compounds usually prefer soft sulphur donors over nitrogen donors. It was reported that, at neutral pH, the rate of guanosine 5'-monophosphate binding to cisplatin increases in the presence of L-methionine.¹¹ This was attributed to the displacement of an ammine ligand by guanosine 5'-monophosphate, since it is labilized by the

trans effect of the sulphur-coordinated L-methionine. In addition, it is widely accepted that sulphur donor ligands are usually co-administered with palladium drugs owing to their important roles in the biological processing of anticancer palladium drugs as protecting agents to reduce the toxicity. The introduction of palladium-sulphur¹² or platinum-carbon^{13,14} bonds results in a significant labilization of the *trans* position, which led to increased reaction rates.

Thiourea is a very convenient and often employed nucleophile used to study ligand substitution reactions in coordination chemistry because of its good solubility, neutral character, and high nucleophilicity. In addition, thiourea is a challenging molecule¹⁵ since it combines the ligand properties of thiolates¹⁶ (π -donor) and thioethers¹⁷ (σ -donor, π -acceptor). Furthermore, it was explored for its applicability as a chemoprotective agent, alleviating the side effect in normal tissue.¹⁸ L-methionine, a protein amino acid, is classified as an essential amino acid for humans, present in blood and available to react with the Pd(II) complex. Also, studies on the interaction of the drug carboplatin with sulphur-containing biomolecules suggest that long-lived Pt(II)-methionine adducts may be important metabolites *in vivo*.¹⁰ Thus, there are several motivations to study the reactions of this complexes (viz., thiourea (tu), and L-methionine (L-Met), as nucleophiles.

Piperazine is an important pharmic intermediate, and piperazine rings are capable of resting in the minor groove of GC base pairs.^{19,20} Moreover, piperazine ring may undergo stacking interactions with the sugar group of DNA, favouring interaction with DNA.

It has been generally accepted that the ultimate target for platinum and palladium anticancer agents is likely to be DNA but the reaction mechanism by which it induces cell death is still to be verified. Recent studies²¹ have revealed that the substitution kinetics of palladium (II) complexes with various ligands in aqueous solution demonstrated a remarkable difference in reactivity between chloro, aqua, and hydroxo complexes, a result that has important consequences for the antitumour activity of related species.

Building on this experience, and in an effort to extend our previous work on the substitution behaviour of $[\text{Pd}(\text{en})(\text{cbdca})]$, $[\text{Pd}(\text{bpy})(\text{cbdca})]$,²² where en = ethane-1,2-diamine, bpy = 2,2-bipyridine and cbdca is cyclobutane 1,1-dicarboxylic acid, and our work on the interaction of $[\text{Pd}(\text{dien})\text{Cl}]^+$ where dien is diethylenetriamine or 1,5-diamino-3-azapentane, with L-cysteine and glutathione,²³ we investigate here the substitution behaviour and associated kinetics of $[\text{Pd}(\text{Pip})\text{Cl}(\text{H}_2\text{O})]^+$ with tu, and L-Met using UV-Vis spectrophotometric and stopped-flow techniques. The investigation is performed as a function of nucleophile concentration and temperature which is important for the general development of new active complexes for cancer therapy.

2. Experimental

2.1 Materials and reagents

Palladium (II) chloride was denoted by Acros Organics. The ligand piperazine as well as the nucleophiles thiourea, and L-methioine, were obtained from Aldrich and Fluka. Nucleophile stock solutions were prepared shortly before use by dissolving the chemicals. All other chemicals were of the highest purity of commercially available and were used without further purification. Ultra-pure water was used in all experiments involving aqueous solutions.

2.2 Preparation of complex solutions

$[\text{Pd}(\text{Pip})\text{Cl}_2]$ was prepared according to the method reported previously²⁴ by heating PdCl_2 (1.773 g, 1 mmol) in 40 ml H_2O and KCl (1.4912 g; 2 mmol) to 70°C for 30 min. The clear solution of $[\text{PdCl}_4]^{2-}$ was cooled to 20°C, filtered and piperazine (0.8614 g,

1 mmol) dissolved in 10 ml H_2O was added drop-wise to the stirred solution. The pH was adjusted to 2–3 by the addition of HClO_4 . A yellow precipitate of $[\text{Pd}(\text{Pip})\text{Cl}_2]$ was formed and stirred for another 2 h at 50°C. Afterwards, the precipitate was filtered off, and washed sequentially with H_2O , ethanol and diethyl ether. Anal. Calcd. for $\text{C}_4\text{H}_{10}\text{N}_2\text{PdCl}_2$: C, 18.22; H, 3.78; N, 10.63%. Found: C, 18.10; H, 3.69; N, 10.86%. Aqueous solutions of the monoqua $[\text{Pd}(\text{Pip})\text{Cl}(\text{H}_2\text{O})]^+$ complex was prepared *in situ* by the addition of slightly less than one mol equivalents of AgNO_3 to a solution of a known amount of the dichloro complex $[\text{Pd}(\text{Pip})\text{Cl}_2]$ and stirred over night. The white precipitate of AgCl that formed was filtered off using a 0.1 μm pore membrane filter. Great care was taken to ensure that the resulting solution was free of Ag^+ ion and that the dichloro complex had been converted completely into the monoqua species. The ionic strength of the solutions was adjusted to 0.1 M with NaNO_3 (Acros, p.a.). The pH of aqueous solutions was measured using a Metrohm 623 pH meter. The pH was carefully monitored during each reaction and will be discussed separately for each reaction studied.

¹HNMR kinetic experiment of the monoqua complex with thiourea was studied on a freshly prepared sample of the reactants to follow the step-wise substitution of the monoqua complex by thiourea. A 17 mM solution of the complex was prepared in 500 μl D_2O approximately 30 min prior to the start of the kinetic experiment; 100 μl of a solution of 70 mM of tu in D_2O (ca. four times excess), was added to initiate the reaction. The spectra were recorded over a period of 28 h. No buffer was added to prevent reaction of complex with buffer components or interfering signals in the observed peak area.

2.3 Instrumentation and measurements

2.3a Potentiometric measurements: Titrations were performed on an automatic titrator (Metrohm SM-Titrino 751). A thermostatted glass cell was used that was equipped with a magnetic stirring system, a combined Metrohm glass electrode (6.0220.100), a thermometric probe, a microburet delivery tube and a salt bridge connected with the reference cell filled with 3 mol·dm⁻³ KCl solution in which Ag/AgCl electrode was dipped. The titroprocessor was calibrated with standard buffer solutions prepared according to NBS specifications.²⁵ The acid dissociation constants of the coordinated water molecule in $[\text{Pd}(\text{pip})(\text{H}_2\text{O})\text{Cl}]^+$ was determined by titrating 0.1 mmol of the complex with NaOH. The titration was carried out at 25°C and 0.1 M

(NaNO_3) ionic strength. A 0.1 M NaOH solution was used as the titrant. The equilibrium constants for the species formed were calculated using the non linear least square computer program MINQUAD-75.²⁶ The stoichiometry and stability constants of the complexes formed were determined by trying various possible composition models. The model selected gave the best statistical fit and was chemically consistent with the titration data without giving any systematic drifts in the magnitudes of various residuals.²⁶ The species distribution diagrams were obtained using the program SPECIES²⁷ under the experimental conditions employed.

2.3b UV-Vis kinetic experiments: UV-Vis spectra were recorded on a Hewlett Packard Diode Array 8452A spectrophotometer equipped with a HP 89090A peltier thermostat ($\pm 0.1^\circ\text{C}$) in a tandem cuvette (optical pathway: 2×0.44 cm). Kinetic measurements of fast reactions were performed on an SX SF-61DX2 kinetic

Table 3. Observed pseudo-first-order rate constants as a function of thiourea concentration; complex, $[\text{Pd}(\text{Pip})\text{Cl}(\text{H}_2\text{O})]^+$, concentration = 0.5 mM, $I = 0.1$, $[\text{tu}] = 5\text{--}30$ mM, $\text{pH} \approx 3.7$.

$T/^\circ\text{C}$	C_{tu}/M	$k_2 \text{ obsd}/\text{s}^{-1}$	$k_3 \text{ obsd}/\text{s}^{-1}$
20	0.005	0.52	0.05
20	0.01	0.90	0.11
20	0.02	1.30	0.18
20	0.03	1.40	0.20
25	0.005	0.80	0.11
25	0.01	1.50	0.15
25	0.02	1.80	0.30
25	0.03	2.10	0.40
30	0.005	1.20	0.14
30	0.01	2.00	0.22
30	0.02	2.40	0.40
30	0.03	2.80	0.60
35	0.005	1.50	0.16
35	0.01	2.30	0.27
35	0.02	3.30	0.50
35	0.03	3.70	0.70

Table 1. Observed pseudo-first-order rate constants as a function of L-methionine concentration; complex, $[\text{Pd}(\text{Pip})\text{Cl}(\text{H}_2\text{O})]^+$, concentration = 0.5 mM, $I = 0.1$, $[\text{L-Met}] = 5\text{--}30$ mM, $\text{pH} \approx 3.5$.

$T/^\circ\text{C}$	$C_{\text{L-Met}}/\text{M}$	$10^2 k_1 \text{ obsd}/\text{s}^{-1}$	$k_2 \text{ obsd}/\text{s}^{-1}$
15	0.005	2.00	0.5
15	0.01	2.50	1.0
15	0.02	2.70	0.9
15	0.03	4.00	1.0
20	0.005	2.36	1.0
20	0.01	3.00	1.5
20	0.02	2.70	2.3
20	0.03	4.00	2.4
25	0.005	2.80	1.9
25	0.01	3.80	2.4
25	0.02	4.80	2.7
25	0.03	5.50	3.7
30	0.005	4.00	2.7
30	0.01	4.80	3.0
30	0.02	5.50	4.0
30	0.03	7.00	4.5

studio in Cairo University, some experiments were measured on 18. MV (Applied Photophysics) stopped flow apparatus, the temperature was controlled throughout all kinetic experiments to an accuracy of $\pm 0.1^\circ\text{C}$. Spectral changes that accompany the reactions were first recorded over the wavelength range of 190–500 nm to establish a suitable wavelength at which the kinetic trace could be followed. The slow reactions were initiated by mixing 900 μl of complex with 900 μl nucleophile solution in a tandem-cuvette, thermostated in a spectrophotometer cell compartment. Complex formation was monitored as an increase in absorbance at 256 nm for L-Met and 310 nm for tu.

The ligand substitution reactions were studied under pseudo-first-order conditions. This was achieved by using at least a 20-fold (1:2 complex formation) or 40-fold (1:4 complex formation) excess of the nucleophile over the complex. All reported rate constants represent an average value of at least three to five independent kinetic runs for each experimental condition and the

Table 2. Rate constants and activation parameters for the reaction of $[\text{Pd}(\text{Pip})\text{Cl}(\text{H}_2\text{O})]^+$ with L-Met. $I = 0.1$ M.

$T/^\circ\text{C}$	$k_1 \times 10^3 \text{ M}^{-1} \text{ s}^{-1}$	$k_{-1} \times 10^2 \text{ M}^{-1} \text{ s}^{-1}$	$k_2 \text{ M}^{-1} \text{ s}^{-1}$	$k_{-2} \text{ M}^{-1} \text{ s}^{-1}$
15	7.32 ± 1.6	1.61 ± 0.31	50.88 ± 12.10	0.44 ± 0.19
20	9.17 ± 1.20	1.92 ± 0.22	58.9 ± 13.97	0.57 ± 0.26
25	10.41 ± 1.53	2.53 ± 0.28	59.76 ± 10.61	0.90 ± 0.20
30	11.35 ± 1.23	3.48 ± 0.23	60.25 ± 7.43	1.32 ± 0.14
$\Delta H^\ddagger/\text{kJ mol}^{-1}$	14.00 ± 0.39	34.95 ± 4.19	13.40 ± 8.03	77.92 ± 5.72
$\Delta S^\ddagger/\text{J K}^{-1} \text{ mol}^{-1}$	-121.1 ± 1.31	81.44 ± 14.1	-166.22 ± 33.32	19.45 ± 19.3

Table 4. Rate constants and activation parameters for the reaction of $[\text{Pd}(\text{Pip})\text{Cl}(\text{H}_2\text{O})]^+$ with tu. $I = 0.1 \text{ M}$.

$T/^\circ\text{C}$	$k_2 \text{ M}^{-1} \text{ s}^{-1}$	$k_{-2} \text{ M}^{-1} \text{ s}^{-1}$	$k_3 \text{ M}^{-1} \text{ s}^{-1}$	$k_{-3} \text{ M}^{-1} \text{ s}^{-1}$
20	30.57 ± 10.12	0.51 ± 0.19	12.20 ± 1.31	0.039 ± 0.024
25	47.20 ± 13.60	0.72 ± 0.25	12.06 ± 0.82	0.040 ± 0.009
30	58.30 ± 14.0	1.15 ± 0.26	19.44 ± 0.52	0.043 ± 0.015
35	86.78 ± 15.75	1.28 ± 0.29	22.33 ± 0.48	0.054 ± 0.009
$\Delta H^\ddagger / \text{kJ mol}^{-1}$	47.17 ± 3.61	44.77 ± 13.20	24.20 ± 6.2	13.60 ± 4.79
$\Delta S^\ddagger / \text{J K}^{-1} \text{ mol}^{-1}$	-55.23 ± 11.97	-97.20 ± 26.52	-141.01 ± 43.8	-225.6 ± 15.8

Figure 1. Concentration distribution of various species as a function of pH in the hydrolysis of $[\text{Pd}(\text{Pip})\text{Cl}(\text{H}_2\text{O})]^+$ complex system (at concentration of 1.25 mL^{-1} for Pd(pip)). **Figure 3.** Concentration and temperature dependence for the first step of reaction of $[\text{Pd}(\text{Pip})\text{Cl}(\text{H}_2\text{O})]^+$ with L-Met. Experimental conditions: $[\text{Pd}(\text{II})] = 0.5 \text{ mM}$; $[\text{L-Met}] = 5\text{--}30 \text{ mM}$; $I = 0.1 \text{ M}$; $\text{pH} = 3.5$; $T = 15\text{--}30^\circ\text{C}$ (every 5°C). **Figure 2.** UV-Vis spectra for the reaction of $[\text{Pd}(\text{Pip})\text{Cl}(\text{H}_2\text{O})]^+$ with L-Met recorded before reaction (1) and after 0.25 (2), 1 (3), 5 (4) 15 (5) and 30 min (6) following mixing of the reagents. Experimental conditions: $[\text{Pd}(\text{II})] = 0.5 \text{ mM}$; $[\text{L-Met}] = 0.01 \text{ M}$; $I = 0.1 \text{ M}$; $T = 25^\circ\text{C}$. Inset: absorbance of curve 1 recorded at 256 nm as a function of time. **Figure 4.** UV-Vis spectra for the reaction of $[\text{Pd}(\text{Pip})\text{Cl}(\text{H}_2\text{O})]^+$ with tu recorded before reaction (1) and after 0.33 (2), 1 (3), 5 (4) 15 (5) and 30 min (6) following mixing of the reagents. Experimental conditions: $[\text{Pd}(\text{II})] = 0.5 \text{ mM}$; $[\text{tu}] = 0.01 \text{ M}$; $I = 0.1 \text{ M}$; $T = 25^\circ\text{C}$.

quoted uncertainties are based on standard deviations. The temperature dependence was studied in the range of 5–35°C. Experimental data are reported in tables 1–4 and are summarized in figures 1–7.

2.3c NMR spectroscopy and elemental analysis:

¹H NMR spectra were recorded on a Bruker Avance DRX 400 WB spectrometer. The measurements were performed with a commercial 5 mm Bruker broad band probe thermostated with a Bruker B-VT 3000 (DRX) variable temperature unit. All chemical shifts were referenced to the solvent signal from D₂O (4.79 ppm). The microchemical analysis of the separated solid complex for C, H and N was performed in the Microanalytical Center, Cairo University. The analyses were performed twice to check the accuracy of the analytical data.

Figure 5. Plot of k_{obs} vs. concentration of thiourea for the first step in the reaction of $[\text{Pd}(\text{Pip})\text{Cl}(\text{H}_2\text{O})]^+$ with tu. Experimental conditions: $[\text{Pd}(\text{II})] = 0.25 \text{ mM}$, $I = 0.1 \text{ M}$, $T = 10^\circ\text{C}$.

Figure 6. Plots of k_{obs} vs. concentration of thiourea for the second and third steps in the reaction of $[\text{Pd}(\text{Pip})\text{Cl}(\text{H}_2\text{O})]^+$ with tu. Experimental conditions: $[\text{Pd}(\text{II})] = 0.25 \text{ mM}$, $I = 0.1 \text{ M}$, $\text{pH} = 3.7$, $T = 25^\circ\text{C}$.

Figure 7. (a) ^1H NMR spectra of the $[\text{Pd}(\text{Pip})\text{Cl}(\text{H}_2\text{O})]^+$ complex before mixing with tu showing the signal of the coordinated Pip at 2.3 ppm. (b) ^1H NMR spectra of the $[\text{Pd}(\text{Pip})\text{Cl}(\text{H}_2\text{O})]^+$ complex after mixing with tu showing the signal of the free Pip at 3.0 ppm after dechelation.

3. Results and discussion

3.1 Acid–base equilibria of $[Pd(Pip)Cl(H_2O)]^+$

The complex $[\text{Pd}(\text{Pip})\text{Cl}(\text{H}_2\text{O})]^+$ ion may undergo protolysis depending on the pH of the solution. Its acid–base chemistry was characterized by fitting the potentiometric data to various acid–base models. The best fit model was found to be consistent with species of the composition 10–1 and 20–1, as given in Eqs. (1) and (2).

The dimmer (20-1) with the single hydroxo-bridge may be formed according to the reaction

The formation constants of the species 10–1 and 20–1 were -6.0 and -1.70 , respectively at 25°C . The equilibrium constant for the dimerization reaction (2) can be calculated with Eq. (3) from formation constants and amounts to,

$$\begin{aligned}\text{Log } K_{\text{dimer}} &= \log \beta_{20-1} - \log \beta_{10-1} \\ &= -1.70 - (-6.0) = 4.30.\end{aligned}\quad (3)$$

The distribution diagram for $[\text{Pd}(\text{Pip})\text{Cl}(\text{H}_2\text{O})]^+$ and its hydrolysed species is shown in figure 1. The dimer with a single hydroxo-bridge reaches the maximum of $\approx 53\%$ at pH 5.3 while the concentration of the mono-hydroxo species (10–1) increases with increase of pH, which is the main species present in solution in the physiological pH range.

3.2 Kinetic studies

The kinetics of the nucleophilic substitution reactions were investigated spectrophotometrically by following the change in absorbance at suitable wavelengths as a function of time using UV-Vis and stopped-flow techniques. The dependence on the nucleophile concentration, and temperature resulted in rate constants and activation parameters ($\Delta H^\ddagger$, $\Delta S^\ddagger$). L-methionine (L-Met) and thiourea (tu) were investigated as nucleophiles because of their different nucleophilicity, steric hindrance, binding properties, and biological relevance (see structures shown in scheme 1). As mentioned above, all kinetic experiments were performed under pseudo-first-order conditions with respect to either the Pd(II) complex or the nucleophile to force the reactions to go to completion.

3.2a Reaction with L-methionine: The reaction of $[\text{Pd}(\text{Pip})\text{Cl}(\text{H}_2\text{O})]^+$ with L-Met leads to spectral changes that are characterized by an increase in absorbance in the UV region of the spectrum viz. a small increase in the maximum at $\approx 300\text{ nm}$ (figure 2), suggesting the formation of a new Pd(II) complex. At pH = 2.0–2.5 the carboxylate group in L-Met is pro-

Scheme 1. Structure of the investigated nucleophiles.

tonated to an extent of Ca. 50% on the basis of the acid dissociation constants for L-Met ($pK_{\text{COOH}} = 2.28$,²⁸ 2.13 ,²⁹ $pK_{\text{NH}_3}^+ = 9.2$ ²⁸), resulting in an overall charge of $+1$. The rest is present as zwitterions. For electrostatic reasons it is reasonable to expect that the reaction will occur between the zwitter ionic form and the positively charged complex. The nucleophilic attack occurs via the sulphur donor of the thioether group. Since it is known that the pK_a values of L-Met decreases significantly upon coordination to a metal ion, we can assume that even if the charged L-Met molecule reacts with the complex, it will deprotonate and the product complex will be of the same overall charge as the reactant mixture. Kinetic measurements were carried out at 256 nm where the absorbance change was more significant, and in excess of the ligand the kinetic traces gave excellent fits to a double exponential function.

Substitution of the ligands of $[\text{Pd}(\text{Pip})\text{Cl}(\text{H}_2\text{O})]^+$ occurs in two subsequent reaction steps. The first step, ascribed to the substitution of the water molecule, is very fast and shows a linear dependence on the concentration of nucleophile, with a second-order rate constant $k_1 = (10.41 \pm 1.53) \times 10^3\text{ M}^{-1}\text{ s}^{-1}$ at 25°C . The second step is much slower and is found to be independent on the concentration of the nucleophile. The independence of the second step on the concentration of L-Met could be attributed to a chelation process. This result is in accordance with the previous work made on some Pt(II) complexes of the type $[\text{PtL}_2\text{X}_2]$ (X = anionic leaving group; L = ammonia, amine, pyridine) with L-Met, where it was proposed that nucleophilic attack *via* the sulphur donor of the thioether group is followed by ring-closure and formation of S,O- or S,N- chelates^{30,31} as shown in scheme 2.

The chelation process should be independent of the L-Met concentration.³¹ The clear concentration independence of the second step on the concentration of L-Met supports our assumption of ring closer and chelation process as reported previously.^{32,33} The reaction was studied as a function of temperature. The

Scheme 2. Second substitution step in the reaction with L-Met involving deprotonation.

observed pseudo-first order rate constants are presented in table 1. The resulting rate constants along with the associated activation parameters are summarized in table 2. The concentration and temperature dependence of the first substitution step is presented in figure 3. This step is proposed to follow an associative mechanism which is confirmed by the relatively large negative value of $\Delta S^\ddagger$ ($-121 \pm 1.31 \text{ J K}^{-1} \text{ mol}^{-1}$) found for this process. The small intercept is ascribed to the back reaction, but is not favourable and has relatively large experimental error limits (viz. $k_{-1} = 2.53 \pm 0.28 \text{ s}^{-1}$ at 25°C). The second step is less efficient mainly due to the steric hindrance caused by coordination of the first L-Met ligand. The second step also exhibits a negative value of $\Delta S^\ddagger$ ($\Delta S^\ddagger = -166.22 \pm 33 \text{ J K}^{-1} \text{ mol}^{-1}$). This is attributed to the deprotonation of the amine during chelate formation. The negative value of the activation entropy for both reaction steps implies an associative substitution mechanism for both reactions.

3.2b Reaction with thiourea: The reaction of $[\text{Pd}(\text{Pip})\text{Cl}(\text{H}_2\text{O})]^+$ with thiourea results in significant changes in the UV-Vis spectra as shown in figure 4. The kinetic measurements were conducted at 310 nm where there is a maximum change in absorbance. The absorbance vs. time traces could only be fitted by three exponentials. Various other kinetic models were tested without leading to a satisfactory fit of the kinetic traces. The effect of temperature on the reaction was studied in the range 10 to 30°C . The observed pseudo-first order rate constants for the second and third steps are presented in table 3. The temperature dependencies allowed for the calculations of enthalpies and entropies of activation by use of Eyring's equation.³⁴ Rate constants and activation parameters derived from these experiments are summarized in table 4. The first step of the reaction assigned to substitution of the water ligand was very fast and could only be measured at low temperature i.e., 10°C with $k_1 = 53\,000 \pm 3000 \text{ M}^{-1} \text{ s}^{-1}$ with an insignificant intercept (figure 5). The second reaction step is slower with $k_2 = 49.96 \pm 13.60 \text{ M}^{-1} \text{ s}^{-1}$ and characterized by a small intercept, $k_{-2} = 0.56 \pm 0.25 \text{ s}^{-1}$, at 25°C . This step was assigned to the substitution of the chloride ligand in the $[\text{Pd}(\text{Pip})\text{Cl}(\text{tu})]^+$ complex. The negative value of the activation entropy ($\Delta S^\ddagger = -55.23 \pm 11.97 \text{ J K}^{-1} \text{ mol}^{-1}$) suggests an associative pathway for this reaction. The intercept associated with this reaction step, although not significant, is assumed to result from a back reaction which, in this case, is not as pronounced as for the reaction with L-Met due to a lower steric hindrance caused by coordinated tu. The concentration dependence of the second and third steps of reaction is presented in

figure 6. Based on the calculated equilibrium constants, we have found that the second substitution step is more efficient for thiourea as a nucleophile than for L-methionine ($K_2 (25^\circ\text{C}) = 82.2 \pm 12 \text{ M}^{-1}$ for tu and $K_2 (25^\circ\text{C}) = 65.5 \pm 7 \text{ M}^{-1}$ for L-Met) ($K_2 = k_2/k_{-2}$). Although the calculated activation entropy for the back reaction has a large error, we can expect an associative substitution mechanism for the displacement of tu, as it was found in the case of L-Met as nucleophile. The third reaction step is ascribed to the subsequent reaction of $[\text{Pd}(\text{Pip})(\text{tu})_2]^{2+}$ with thiourea, as a result of the strong *trans* effect of tu, in which the piperazine ligand is dechelated and displaced by tu (see Eqs. (4)–(7))

The amine labilization process has frequently been observed for reactions of such complexes with sulphur containing nucleophiles.^{22,32} This step is much slower ($k_3 = 15.52 \pm 0.82 \text{ M}^{-1} \text{ s}^{-1}$ at 25°C) than the substitution of the water and chloride ligands. The release of the piperazine ligand during the substitution process was also confirmed by ^1H NMR technique. Figure 7 shows ^1H NMR spectra over a time range of 28 h, showing the difference in the chemical shifts between bidentate coordinated, ring-opened and free Pip ligand. The signals of the coordinated diamine ligand, N-CH₂ (2.3 ppm) (figure 7a) disappears directly after mixing with tu and new signal corresponding to the free piperazine appears downfield (3.0 ppm) (figure 7b). The continuous shift of the signals indicates the change in pH of the solution due to the release of

the diamine ligand. We also observed for a more concentrated sample, a change in colour from yellowish to red directly upon addition of thiourea, which is in good agreement with earlier findings for related systems.³⁵

The linear dependence of the observed rate constant on the thiourea concentration suggests a direct substitution pathway (Eq. 4). The intercept of $0.04 \pm 0.009 \text{ s}^{-1}$ can either be attributed to the contribution from the reverse reaction (k_{-3}) or to the contribution from a parallel slow solvolysis pathway (Eq. 6) that is followed by a rapid displacement of coordinated ligand by tu in Eq. 7. Both k_3 and k_{-3} are characterized by a negative activation entropy which points to the associative character of these reactions. The third substitution step of the reaction with tu shows a more negative activation entropy than that for the second step (table 2). This can be explained on the basis of the liberation of the piperazine ligand which results in charge creation, protonation of the diamine ligand, and generation of OH^- . The new complex, suggested to be $[\text{Pd}(\text{Pip-N})(\text{tu})_3]^{2+}$, is not very stable and undergoes a further reaction with tu as indicated by further changes in the UV-Vis spectra as shown in figure 4, on longer reaction times to form $[\text{Pd}(\text{tu})_4]^{2+}$.

4. Conclusions

The interaction of a palladium (II) complex towards bio-relevant thiol ligands may throw some light on the bioinorganic chemistry of palladium (II). The present study clearly demonstrates that the π -accepting property of the piperazine ring increases the electrophilicity on the Pd(II) metal centre, which results in increasing reaction rate for nucleophilic substitution reactions. This can be observed by the higher rate of substitution with tu compared to that obtained in our previous work with ethylenediamine complex.²² Furthermore, the strong *trans* effect of piperazine complex facilitates the labilizing effect of strong donor nucleophiles.

The reaction with L-methionine proceeds first via substitution of a water molecule by sulphur, followed by an intramolecular ring-closure step via nitrogen, and thus the second step is independent of the nucleophile concentration. This is supported by the value of the rate constant (k_2) for the second step which was actually found to be independent of the ligand concentration. The second step exhibits a very large negative value of $\Delta S^\ddagger$. This is attributed to the deprotonation of the amine during chelate formation. Whereas, the reaction with tu proceeds via three steps that are all dependent on the nucleophile concentration. The third step leads to displacement of the Pip ligand and formation of the

fully substituted Pd(II) complex as previously found for other Pd(II) complexes.^{22,32,33} The release of the Pip ligand during the substitution process with tu is confirmed by recording the ^1H NMR spectra over a time period of 28 h. The mechanism of the substitution reactions is associative in nature as supported by the large negative values of $\Delta S^\ddagger$.

In the present study, tu was found to be a stronger nucleophile than L-Met. The extent of the second step, is governed by the steric hindrance caused by coordination of the first nucleophile. Therefore, the second step of nucleophilic substitution of the less bulky thiourea ligand would proceed more easily. Furthermore, the piperazine chelate ligand also introduces the possibility of effective interaction into DNA, an aspect that could be of significance for the actual activity of this complex through the modification of the coordination sphere, and it is our goal to develop potential antitumour complexes of varying reactivity that can be employed for the different types of tumours treated in different ways through the systematic tuning of spectator ligands.

In conclusion, our findings point to an important role of S-donor ligands as strong and efficient nucleophiles which can easily compete with DNA for binding to Pd(II) or Pt(II) complexes^{31,36} and in that way deactivate their anticancer activity. In addition, it supports the hypothesis of a drug reservoir mechanism in which the palladium–sulphur adduct is an intermediate for palladium binding to DNA.³⁷

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