Kinetics and mechanism of interaction of some bioactive ligands with *cis*-diaqua(*cis*-1,2-diaminocyclohexane)platinum(II) in aqueous medium

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Abstract. The substitution reaction of $cis\text{-[Pt}(cis\text{-dach})(\text{H}_2\text{O})_2]^{2+}$ (where ‘dach’ is *cis*-1,2-diaminocyclohexane) with 2-thiouracil (S, N), 1,2-cyclohexanedionedioxime (N, N) and acetylacetone (O, O) were studied in aqueous solution in 0.10 M NaClO₄ under pseudo-first order conditions as a functions of concentration, pH and temperature using UV-Vis spectrophotometry. The substitution reaction proceeds via rapid outer sphere association complex formation, followed by two slow consecutive steps. The first of these involves ligand-assisted deaquation, while second involves chelation as the second aqua ligand is displaced. The association equilibrium constant (K_E) for the outer sphere complex formation has been evaluated together with rate constants for the two subsequent steps. The rate constants increase with increasing ligand concentration and the evaluated activation parameters for all reactions suggest an associative substitution mechanism for both the aqua ligand substitution processes. The product of the reaction has been characterized by IR, NMR and ESI-MS spectral analysis; which throws more light on the mechanistic behaviour of platinum(II) antitumour complexes.

Keywords. Platinum(II); diaminocyclohexane; kinetics; bioactivity.

1. Introduction

Cisplatin (CDDP) is one of the most effective anticancer drugs currently used worldwide in the treatment of testicular, ovarian, and other tumours.^{1,2} The usefulness of this transition metal complex is limited, however, by its limited spectrum of responsive tumours, rather severe host toxicities such as nausea and vomiting, nephrotoxicity, neurotoxicity and the potential to induce resistance in otherwise responsive tumour types.³ In addition, cisplatin has been classified as a probable human carcinogen.⁴ In the search for cisplatin analogs with a better toxicity profile and an improved spectrum of activity, a large number of platinum (Pt) derivatives have been synthesized and investigated.^{5–7} One of the most promising second generation platinum complexes was *cis*-dichloro(1,2-diaminocyclohexane)platinum(II).⁸ The biological properties of this complex which included excellent antitumour activity, little or no nephrotoxicity and lack of cross resistance with CDDP.⁸ Much progress has been made in elucidating its mode of action, and

many details of the mechanism by which platinum-based drugs kill cancer cells are now well-established. Biological and chemical experiments have indicated that the interaction of cisplatin with DNA is responsible for its antineoplastic activity.^{9,10} Nevertheless, some essential chemical processes, related to what happens before the cisplatin reaches the DNA, generally considered its final target, are still to be identified. Among these processes, the best known is the formation of aqua species, the main reaction of activation of the drug which occurs in the cytoplasmic compartment by hydrolysis of the chloride ligands. However, many other non-genomic biomolecules could be potential targets for platinum. Sulphur-rich biomolecules, including free amino acids (cysteine and methionine), oligopeptides (glutathione), and proteins represent good targets for a soft metal such as Pt(II).^{11–13} Moreover, the need to improve the cisplatin clinical protocol drives much research into better understanding of its antitumour activity mechanism. On the other hand, in order to overcome acquired cellular resistance to cisplatin, much effort is currently devoted to the discovery of new Pt anticancer drugs.

We reported the kinetics and mechanism of the complex formation reaction of $cis\text{-[Pt}(cis\text{-dach})(\text{H}_2\text{O})_2]^{2+}$

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(where 'dach' is *cis*-1,2-diaminocyclohexane) with some biologically relevant nitrogen, oxygen and sulphur donor nucleophiles (2-thiouracil, 1,2-cyclohexanedionedioxime and acetylacetone). The aqua complex studied is more reactive than the corresponding chloro complex. The coordinated water molecule on Pt(II) centre has been shown to be very labile and can more easily be substituted than a stronger nucleophilic group such as chloride. These nucleophiles were investigated because of their different nucleophilicity, steric hindrance, binding properties and biological relevance. This study is of fundamental importance to unravel the mode of action of this class of compounds and it throws more light on the kinetic and mechanistic behaviour of platinum anti-tumour complexes. The goal is to contribute to the elucidation of the whole mechanism employed by these compounds to reach the biological target.

2. Experimental

The substrate chloro complex *cis*-dichloro-(*cis*-dach)platinum(II) was prepared according to literature method.¹⁴ The reactant complex *cis*-[Pt(*cis*-dach)(OH₂)₂](ClO₄)₂ (**1**) (dach = diaminocyclohexane) was prepared from *cis*-dichloro-(*cis*-dach)platinum(II) by hydrolysis in the presence of two molar equivalent of silver perchlorate. The chloro compound spread over the aqueous solution of silver perchlorate and mixture was kept for 24 h and then filtered to remove AgCl. The diaqua complex was then characterized spectrophotometrically. The pHs of the solutions were maintained at pH (4.0), so that perchlorate salt exists as the diaqua species. The products of the reaction between (**1**) and 2-thiouracil (L¹H) or 1,2-cyclohexanedionedioxime (L²H) or acetylacetone (L³H) were prepared by mixing the reagents at pH 4.0 in different molar ratio: viz 1:1, 1:2, 1:3, 1:4 and 1:5 and thermostating the mixture at 60°C for 48 h. The absorption spectra of the resulting solutions were recorded and all are found to exhibit almost identical absorbances at 327, 298 and 308 nm wavelength for L¹H, L²H and L³H, respectively. The spectral differences between the product complexes and the substrate complex are shown in figure 1. The relative $\lambda_{\max}$ positions of the LH-substituted products are in accordance with the polarizability of the ligands.

The compositions of the products in the reaction mixtures were determined by Job's method of continuous variation (figure 2). The metal: ligand ratios were found to be 1:1 for L¹H, L²H and L³H.

Complex (**1**) and 2-thiouracil (L¹H) were mixed in 1:1 molar ratio at pH 4.0 and a yellowish product was isolated. The IR spectrum of the yellowish product in

Figure 1. Spectra of the starting complex (**1**), L¹H substituted complex (**2**) L²H substituted complex (**3**) and L³H substituted complex (**4**). [complex (**1**)] = 2.00×10^{-4} mol dm⁻³, [L¹H], [L²H] and [L³H] = 4.00×10^{-3} mol dm⁻³, cell used = 1 cm quartz, pH = 4.0, and ionic strength = 0.10 mol dm⁻³ NaClO₄.

Figure 2. Job's plot for the reaction between complex (**1**) and L³H at pH = 4.0 and ionic strength = 0.10 mol dm⁻³ NaClO₄.

the KBr disc shows a strong band at 3411 together with medium band at 530 cm⁻¹. The IR spectrum of L¹H does not show any ν (SH) band; hence in the solid state ligand exists mainly in the keto-thione form. The broad bands at 3424 and 3411 cm⁻¹ in L¹H and its substituted complex, respectively, are due to ν (OH) of water molecules and hydrogen bonds of the type N-H...O. The ν (NH) bands at 3134 and 3049 cm⁻¹ are strongly affected on complexation to Pt(II); the presence of only one band at 3236 cm⁻¹ indicates NH group participates in bond formation with metal ion.¹⁵ The ν (C = O) and ν (C = N) bands of L¹H at 1702 and 1629 cm⁻¹, respectively, are affected on complexation. With respect to the free L¹H, the band at 1239 cm⁻¹ corresponding to stretching of the most ν (C = S) character shifts to lower wave number in product, which indicate the stronger coordination of the sulphur atom

to the Pt(II). The band at 530 cm^{-1} is also assigned to $\nu(\text{Pt-N})$ bond formation.¹⁶ The spectrum suggests that the final product is an (S, N_3) coordinated adjacent chelate and the 2-thiouracil behave as a bidentate ligand in the experimental pH.¹⁷ Similarly, the presence of moderately strong band at 636 cm^{-1} for brown product of complex (**I**) and L^3H indicates the formation of Pt–O bond.¹⁸

The aqueous solution of $\text{cis-[Pt(cis-dach)(OH}_2\text{)}_2\text{]}^{2+}$ and L^1H were mixed in a 1:1 molar ratio and the

mixture was thermostated at 60°C for 48 h and used for ESI-MS measurement. The ESI mass spectrum of the resulting solution is shown in figure 3.

It is clear from this spectrum that the ion at m/z 436.20 (minor peak) has become the precursor ion species in the mixture solution and this is tentatively attributed to $(\text{L}^1\text{H} + \text{Pt(II)} + \text{dach} - \text{H}^+)^+$. The peak at 450.25 indicates the formation of the product $(\text{L}^2\text{H} + \text{Pt(II)} + \text{dach} - \text{H}^+)^+$ (figure 4). Similarly, the major peak at 408.3070 assigned to the $(\text{L}^3\text{H} + \text{Pt(II)} + \text{dach})$

Figure 3. ESI- mass spectrum of the product for complex (**I**) and L^1H at pH 4.0 in aqueous medium and at ionic strength $0.1\text{ mol dm}^{-3}\text{ NaClO}_4$.

Figure 4. ESI- mass spectrum of the product for complex (**I**) and L^2H at pH 4.0 in aqueous medium and at ionic strength $0.1\text{ mol dm}^{-3}\text{ NaClO}_4$.

Figure 5. ESI-mass spectrum of the product for complex (**I**) and L^3H at pH 4.0 in aqueous medium and at ionic strength $0.1 \text{ mol dm}^{-3} \text{ NaClO}_4$.

$-H^+)^+$; the relative abundance of isotope peaks match the expected values, i.e., m/z 407.3076, m/z 409.3076 and m/z 411.3124 (figure 5).¹⁹

The 1H NMR spectrum of L^1H exhibits only two doublets, centred at δ 7.5 and 6.08 ppm, assigned to C_5 and C_6 protons, respectively. The amide protons are not detected in the spectrum and data suggest that the ligand exists in rapid equilibrium. If we look at the 1H NMR of the platinum complex, the peak of the C_5 and C_6 proton is shifted significantly. This clearly indicates the coordination of the L^1H with metal centre (figure 6). Thus the structures proposed here for product ion species, deduced from ESI-mass and NMR spectra are consistent with those derived from other experimental methods.

2.1 Physical measurement

All the spectral scanning and kinetic measurements were done in a Shimadzu UV 2450 spectrophotometer attached to a thermoelectric cell temperature controller (model TCC-240A with an accuracy of $\pm 0.1^\circ\text{C}$). IR Spectra (KBr disc, $4000\text{--}350 \text{ cm}^{-1}$) were measured in Perkin-Elmer FTIR model RX1 Infrared spectrophotometer. ESI-mass spectra recorded using a micromass Q-Tof microTM mass spectrometer in +ve ion mode. 1H NMR spectrum was recorded using Bruker AVANCE III 400 MHz spectrometer. The pHs of the solutions were adjusted by adding NaOH/ $HClO_4$ and the measurements were carried out with the help of a Sartorius Digital pH meter (model PB11) with an accuracy of

± 0.01 units. Doubly distilled water was used to prepare all the solution. All other chemicals used were of AR grade. The reactions were carried out at constant ionic strength ($0.10 \text{ mol dm}^{-3} \text{ NaClO}_4$).

2.2 Kinetic measurements

The kinetics of substitution of coordinated water molecule was followed spectrophotometrically by following the change in absorption at suitable wavelengths as a function of time. The working wavelengths were determined by recording the spectra of the reaction mixture over the wavelength range between 200 and 500 nm and are given in figure 1. All kinetic experiments were performed under pseudo-first-order conditions. The reactions were initiated by mixing equal volume of complex and ligand solutions (1.5 mL) in the quartz cuvette. The absorption due to ligands was subtracted by using 1:1 (molar ratio) ligand:water mixture in the reference cell. The concentration of ligand solution was always large enough (at least a ten-fold excess) to provide pseudo-first-order conditions. A typical plot of $\ln(A_\infty - A_t)$ (where A_t and A_∞ are absorbances at time (t) and after completion of reaction) against time (figure 7) is found to be nonlinear; it is curved at initial stage and subsequently of constant slope indicating that the reaction proceeds via two consecutive steps. From the limiting linear portion of $\ln(A_\infty - A_t)$ versus time (t) curve, $k_{2(\text{obs})}$ were obtained. The $k_{1(\text{obs})}$ values were obtained from the plots of $\ln \Delta$ versus time (t) where time (t) is small (figure 8). Origin software was

Figure 6. ^1H NMR spectra of 2-thiouracil and its substituted product.

used for computational works. Rate data, represented as an average of duplicate runs, are reproducible within $\pm 4\%$.

3. Results and discussion

The pK_a values of the ligands L^1H , L^2H and L^3H are 9.78 and 12.7,²⁰ 12.0²¹ and 8.76,²² respectively, at 25°C . So at pH 4.0, ligands remain in the neutral form, which participate in the reaction. The pK_1 and pK_2 (6.25 and 7.80) for $cis\text{-(diaqua}(cis\text{-1,2-diaminocyclohexane)platinum(II))$ has been evaluated

by Irving–Rossotti titration technique.²³ Therefore we can assume that at pH 4.0 the reactant exists as the diaqua ion. The reactions followed two-step consecutive process; the first step is dependent on ligand concentration whereas the second is the independent of ligand concentration. In the first step one aqua ligand was replaced from $cis\text{-[Pt}(cis\text{-dach})(\text{OH}_2)_2]^{2+}$ by LH . The second is a slower step, where another aqua ligand is substituted. This is the ring closure step. The rate constant for such a process can be evaluated by assuming the following scheme

Figure 7. A typical kinetic plot of $\ln(A_{\infty} - A_t)$ versus time (t). [complex (**I**)] = $2 \times 10^{-4} \text{ mol dm}^{-3}$; [L^2H] = $2 \times 10^{-3} \text{ mol dm}^{-3}$; pH = 4.0; $\mu = 0.1 \text{ mol dm}^{-3} \text{ NaClO}_4$ and temperature = 35°C .

Figure 8. A typical kinetic plot of $\ln\Delta$ versus time (t). [complex (**I**)] = $2.0 \times 10^{-4} \text{ mol dm}^{-3}$; [L^2H] = $2.0 \times 10^{-3} \text{ mol dm}^{-3}$; pH = 4.0; $\mu = 0.1 \text{ mol dm}^{-3} \text{ NaClO}_4$ and temperature = 35°C .

where A is the diaqua species (**I**); B is the single substituted intermediate and C is the final product $[\text{Pt}(\text{cis-dach})(\text{L})]^+$. Formation of C from B is predominant after some time has elapsed.

3.1 Calculation of k_1 for $A \rightarrow B$ step

The rate constant $k_{1(\text{obs})}$ for the $A \rightarrow B$ step can be evaluated by the method of Wey and Hamm²⁴ using the usual consecutive rate law.²⁵ A similar procedure is applied for all ligands in the concentration range of $0.002\text{--}0.006 \text{ mol dm}^{-3}$ at constant complex (**I**) concentration of $0.0002 \text{ mol dm}^{-3}$ at pH 4.0 and at 30, 35, 40, 45 and 50°C , respectively. The $k_{1(\text{obs})}$ values thus obtained are linearly dependent on the studied concentration range. The $k_{1(\text{obs})}$ values for different ligand concentrations at different temperatures are given in table 1. The ligand concentration dependence of $k_{1(\text{obs})}$ values can be explained in terms of rapid formation of an outer sphere association complex between the reactant complex (**I**) and the sulphur end of neutral form of L^1H in the $A \rightarrow B$ stage. The rate increases with increase in ligand concentration and reaches a limiting value (figure 9), which is probable due to the completion of the outer sphere association complex formation. Since the metal ion reacts with immediate environment, further change in ligand concentration beyond the saturation point will not affect the reaction rate and a gradual approach towards limiting rate is observed. At this stage, the interchange of the ligands from outer sphere

Table 1. $10^3 k_{1(\text{obs})} (\text{s}^{-1})$ values for ligand concentrations at different temperatures. [complex (**I**)] = $2.00 \times 10^{-4} \text{ mol dm}^{-3}$, pH = 4.0, ionic strength = $0.1 \text{ mol dm}^{-3} \text{ NaClO}_4$.

Ligand	Temperature ($\pm 0.1^\circ\text{C}$)	$10^3 \times [\text{Ligand}] (\text{mol dm}^{-3})$				
		2.00	3.00	4.00	5.00	6.00
L^1H	30	2.60	3.33	3.98	4.43	4.76
	35	3.74	4.70	5.37	5.74	6.18
	40	4.89	6.04	6.69	7.18	7.61
	45	5.72	6.89	7.68	8.15	8.60
	50	6.18	7.35	8.22	8.72	9.18
L^2H	30	0.38	0.49	0.59	0.69	0.76
	35	0.53	0.71	0.83	0.97	1.03
	40	0.78	0.97	1.18	1.36	1.49
	45	1.16	1.46	1.70	1.89	2.11
	50	1.75	2.16	2.47	2.67	2.85
L^3H	30	0.33	0.43	0.51	0.58	0.65
	35	0.47	0.60	0.71	0.80	0.88
	40	0.66	0.84	0.97	1.11	1.21
	45	0.93	1.18	1.36	1.50	1.67
	50	1.49	1.83	2.08	2.27	2.43

to the inner sphere occurs. The following scheme can be proposed;

where L–LH indicate bidentate form of L¹H, L²H and L³H. Based on the above equations a rate expression (7) can be derived for the A→B step:

$$\frac{dB}{dt} = \frac{k_1 K_E [\text{Pt}(\text{cis}-\text{dach})(\text{H}_2\text{O})_2^{2+}]_{\text{total}} [\text{LH}]}{1 + K_E [\text{LH}]} \quad (4)$$

or,

$$\frac{dB}{dt} = k_{1(\text{obs})} [\text{Pt}(\text{cis}-\text{dach})(\text{H}_2\text{O})_2^{2+}]_{\text{total}} \quad (5)$$

where [Pt(*cis*-dach)(H₂O)₂]²⁺_{total} is the total concentration of the complex, [LH] is the concentration of the bio-active ligands. Hence we can write;

$$k_{1(\text{obs})} = \frac{k_1 K_E [\text{LH}]}{1 + K_E [\text{LH}]} \quad (6)$$

where k₁ is the rate constant for conversion of outer sphere complex to inner sphere complex and K_E is the outer sphere association equilibrium constant. Equation (6) can be rearranged:

$$1/k_{1(\text{obs})} = 1/k_1 + 1/k_1 K_E [\text{LH}]. \quad (7)$$

A plot of 1/k₁₍obs) versus 1/[LH] should be linear with an intercept of 1/k₁ and slope 1/k₁K_E. This was found to be so, at all temperature studied (figure 10). The k₁ and K_E values obtained from intercept and from slope to intercept ratios are included in table 2.

Figure 9. Plots of $10^3 k_{1(\text{obs})}(\text{s}^{-1})$ versus $[\text{L}^1\text{H}]$ at different temperatures; A = 30°C, B = 35°C, C = 40°C, D = 45°C and E = 50°C; pH 4.0 and ionic strength = 0.10 mol dm⁻³ NaClO₄.

Figure 10. Plots of $1/k_{1(\text{obs})}(\text{s})$ versus $1/[\text{L}^1\text{H}]$ at different temperatures; A = 30°C, B = 35°C, C = 40°C, D = 45°C and E = 50°C; pH 4.0 and ionic strength = 0.10 mol dm⁻³ NaClO₄.

Table 2. $10^5 k_{2(\text{obs})}$ (s^{-1}) values for ligand concentrations at different temperatures. [complex (*I*)] = 2.00×10^{-4} mol dm $^{-3}$, pH = 4.0, ionic strength = 0.1 mol dm $^{-3}$ NaClO $_4$.

Ligand	Temperature ($\pm 0.1^\circ\text{C}$)	$10^3 \times [\text{Ligand}]$ (mol dm $^{-3}$)				
		2.00	3.00	4.00	5.00	6.00
L ¹ H	30	3.61	3.64	3.63	3.66	3.64
	35	4.41	4.40	4.40	4.41	4.42
	40	5.09	5.11	5.10	5.10	5.12
	45	6.13	6.15	6.13	6.10	6.16
	50	7.20	7.20	7.21	7.22	7.19
L ² H	30	1.88	1.86	1.87	1.88	1.89
	35	2.34	2.35	2.35	2.37	2.36
	40	3.22	3.24	3.25	3.26	3.24
	45	4.49	4.47	4.48	4.49	4.45
	50	5.55	5.53	5.52	5.50	5.51
L ³ H	30	1.70	1.73	1.72	1.72	1.74
	35	2.32	2.33	2.30	2.35	2.33
	40	3.16	3.17	3.15	3.14	3.18
	45	4.23	4.28	4.25	4.25	4.24
	50	5.32	5.33	5.34	5.31	5.34

3.2 Calculation of k_2 for the B→C step

The B→C step is the ring closure step in which the second donor site of LH binds the metal centre. Due to the steric hindrance, this chelation step is slower and independent of ligand concentration variation. At each temperature, the k_2 values were calculated from the limiting linear portion (where time (*t*) is large) of the $\ln(A_\infty - A_t)$ versus time (*t*) curves (figure 7) and are given in tables 2

and 3. Unlike k_1 , k_2 was found to be independent of ligand concentration at each of the temperature studied.

3.3 Effect of pH on reaction rate

The reaction was studied at five different pH values. At a fixed 2.00×10^{-4} mol dm $^{-3}$ [complex (*I*)], 4.00×10^{-3} mol dm $^{-3}$ [LH] and 0.10 mol dm $^{-3}$ NaClO $_4$ ionic

Table 3. $10^3 k_1$, $10^5 k_2$ and K_E values for different ligands at different temperatures, [complex (*I*)] = 2.00×10^{-4} mol dm $^{-3}$, pH = 4.0, ionic strength = 0.1 mol dm $^{-3}$ NaClO $_4$.

Ligand	Temperature ($\pm 0.1^\circ\text{C}$)	$10^3 k_1$ (s^{-1})	$10^5 k_2$ (s^{-1})	K_E (dm $^{-3}$ mol $^{-1}$)
L ¹ H	30	8.11	3.64	235
	35	9.05	4.41	355
	40	10.42	5.10	447
	45	11.39	6.13	508
	50	12.01	7.20	531
L ² H	30	1.47	1.88	171
	35	2.02	2.35	179
	40	2.70	3.24	195
	45	3.38	4.48	258
	50	4.15	5.52	364
L ³ H	30	1.20	1.72	188
	35	1.53	2.33	214
	40	2.01	3.16	249
	45	2.64	4.25	277
	50	3.51	5.33	368

Table 4. $10^3 k_{1(\text{obs})}$ and $10^5 k_{2(\text{obs})}$ values for different ligands at different pH, [complex (*I*)] = 2.00×10^{-4} mol dm⁻³, [LH] = 4.00×10^{-3} mol dm⁻³, ionic strength = 0.1 mol dm⁻³ NaClO₄.

Ligand	pH (± 0.01)	$10^3 k_1$ (s ⁻¹)	$10^5 k_2$ (s ⁻¹)
L ¹ H	3.0	6.65	3.17
	3.5	6.66	4.25
	4.0	6.69	5.10
	4.5	6.69	6.86
	5.0	6.71	8.94
L ² H	3.0	1.17	1.69
	3.5	1.18	2.40
	4.0	1.18	3.24
	4.5	1.19	4.51
	5.0	1.19	6.01
L ³ H	3.0	0.95	2.12
	3.5	0.96	2.73
	4.0	0.97	3.16
	4.5	0.97	4.06
	5.0	0.98	5.13

strength, the $10^3 k_{1(\text{obs})}$ and $10^5 k_{2(\text{obs})}$ values are given in table 4 at 40°C. The $k_{2(\text{obs})}$ values were only found to increase with the increase in pH in the studied pH range. The enhancement in rate may be explained by the acid dissociation equilibria of the ligands and the complex. Within our studied pH range the ligands remain unchanged, so the effects of pH on rate are therefore due to the change in reactive forms of the reacting complex. The complex changes its form, from aqua to hydroxo-aqua. The hydroxo species is more reactive due to the well-known labilising effect of the -OH group via its π -bonding ability and strong electromeric effect.

Figure 11. Plots of pH versus $10^5 k_{2(\text{obs})}$ for the three ligands.

The pH dependence of $k_{2(\text{obs})}$ can also be readily interpreted in terms of the changes with pH in fractional populations of species involved in the respective reactions.²⁶ From the plot of pH versus $10^5 k_{2(\text{obs})}$ (figure 11) for three ligands shows that after pH 4.0 all the ligands have greater sensitivity with pH which is consistent with the pK_a value of the mono substituted complex. Notwithstanding in the present kinetic runs, the substitution reactions were followed at a constant pH of 4.0 to avoid complication caused by adding an additional parameter of $[H^+]$ to the rate equation.

3.4 Effect of temperature on reaction rate

The reaction was studied at five different temperatures for different ligand concentrations and the anation rate constants for both $A \rightarrow B$ (k_1) and $B \rightarrow C$ (k_2) steps are given in table 3. The activation parameters calculated from Eyring plots (figures 12 and 13) are given in

Figure 12. Eyring plots of L¹H, L²H and L³H for k_1 .

Figure 13. Eyring plots of L¹H, L²H and L³H for k_2 .

Table 5. Activation parameters for analogous systems.

Systems	$\Delta H_1^\ddagger$ (kJmol ⁻¹)	$\Delta S_1^\ddagger$ (JK ⁻¹ mol ⁻¹)	$\Delta H_2^\ddagger$ (kJmol ⁻¹)	$\Delta S_2^\ddagger$ (JK ⁻¹ mol ⁻¹)	Refs.
<i>Cis</i> -[Pt(<i>cis</i> -dach) (H ₂ O) ₂] ²⁺					
/Glutathione	32.9 ± 1.3	-187.2 ± 4.2	30.5 ± 0.1	-223.1 ± 0.3	[27]
/Et ₂ DTC	66.8 ± 3.7	-81 ± 12	95.1 ± 2.8	-34.4 ± 9.1	[28]
/DL-Penicillamine	36.1 ± 4.1	-175 ± 12	44.4 ± 1.1	-189 ± 3	[25]
/L ¹ H	14.1 ± 1.0	-239 ± 3	24.9 ± 1.2	-248 ± 4	[**]
/L ² H	39.7 ± 1.5	-168 ± 5	43.0 ± 1.5	-194 ± 5	[**]
/L ³ H	41.1 ± 2.0	-166 ± 6	44.1 ± 0.7	-191 ± 2.1	[**]

**This work

table 5 and compared with those for analogous systems involving the substitution in square planar platinum(II) complexes.

4. Mechanism and conclusion

All the ligands exist as neutral molecules at experimental pH 4.0. Job's method of continuous variation indicate 1:1 molar ratio and the IR spectra of the solid products suggest that the ligands behaved as bidentate ligand. Finally, ¹H NMR and ESI-mass spectra provide a qualitative picture of the composition i.e., the ligational sites of the nucleophiles. Thus, the mechanism of substitution of aqua ligands in *cis*-diaqua(*cis*-1,2-diaminocyclohexane)platinum(II) ion can be explained in terms of rapid outer sphere association complex formation, followed by two consecutive steps; the first is dependent on ligand concentration and second is the chelation i.e., ring closure step, which is slower than the first step and independent of ligand concentration (figure 14).

The rate constant 'k₁' for the ligands 2-thiouracil (S, N), 1,2-cyclohexanedionedioxime (N, N) and acetylacetone (O, O) of different donor sets, can be rearranged as

The sulphur end of 2-thiouracil is a soft donor centre and has large affinity for the soft Pt centre. The sulphur containing ligands have higher reactivity than ligands containing no sulphur atom. This is due to ability of sulphur atom to function both as σ -donor and as π -acceptor. Aside this, Pt(II) is soft acid and soft-soft interaction initiates the preference of Pt(II) to sulphur ligands. The cyclization process occurs with deprotonation, a phenomenon, which is dependent on the pH and on the nature of the metal ion. At pH 4.0, deprotonation is difficult to occur resulting the reaction becomes

Figure 14. Plausible mechanism for the substitution of the aqua ligand from *cis*-[Pt(*cis*-dach)(H₂O)₂]²⁺ by L²H.

slower. With 1,2-cyclohexanedionedioxime, the presence of polar side chain probably helps deprotonation by lowering the energy barrier through solvation. Moreover, owing to better solvation of transition state caused

by the presence of hydrophilic site in case of 1,2-cyclohexanedionedioxime, shows higher rate than that of acetylacetone. Therefore Pt(II) has greater affinity for nitrogen centre than that of oxygen.

The trend in the reactivity of the ligands can also be seen in the activation enthalpies for the reactions involving the complex (**I**). The higher the nucleophilicity of the ligand, the smaller the activation enthalpy because of stabilization of the transition state.

The enthalpies of activation ($\Delta H_1^\ddagger$ and $\Delta H_2^\ddagger$) values and negative values of entropies of activation ($\Delta S_1^\ddagger$ and $\Delta S_2^\ddagger$) imply a good degree of ligand participation in the transition state (table 3). The positive enthalpy change for breaking the M–OH₂ bond is partially compensated by the formation of M–L bond in the transition state. The participation of LH in the transition state results in a more compact state and a negative $\Delta S^\ddagger$ value is obtained. Further $\Delta S_2^\ddagger$ is more negative than $\Delta S_1^\ddagger$, which suggest that compactness has already been achieved in B and the transformation of B to C is only the replacement of another aqua ligand through chelation.

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