Kinetics of substitution of aqua ligands from cis-diaqua(cis-1,2-diaminocyclohexane)platinum(II) perchlorate by glutathione in aqueous medium

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The kinetics of the interaction of glutathione (reduced) (GSH) with [Pt(DACH)(H₂O)₂]²⁺ (DACH = cis-1,2-diaminocyclohexane) have been studied spectrophotometrically as a function of [Pt(DACH)(H₂O)₂]²⁺, [glutathione] and temperature at a particular pH (4.0). The reaction has been found to proceed via rapid oustersphere association complex formation followed by two slow consecutive steps. The first step involves the transformation of the oustersphere complex into the inner sphere complex containing Pt-S bond and one aqua ligand while the second step involves chelation when the second aqua ligand is displaced. The association equilibrium constant (K₂) and the two rate constants k₁ and k₂ have been evaluated. Activation parameters for both the steps have been calculated using Eyring equation (ΔH° = 32.87 ± 1.31 kJ mol⁻¹, ΔS° = -187.24 ± 4.2 J K⁻¹ mol⁻¹, ΔH₂ = 30.50 ± 0.09 kJ mol⁻¹, ΔS₂ = -223.05 ± 0.28 J K⁻¹ mol⁻¹). The low enthalpy of activation and large negative values of entropy of activation indicate an associative mode of activation for both steps.

Materials and Methods

The reactant complex (complex I) was prepared from cis-dichloro- (DACH)platinum(II) by hydrolysis reaction in presence of two molar quantities of silver perchlorate. The chloro compound was spread over aqueous solution of silver perchlorate and the mixture was kept in dark for 24 hour and then filtered to remove AgCl. The diqua compound was then characterised spectrophotometrically. The chloro complex, cis-dichloro (DACH)platinum(II) was prepared by literature method. The product of the reaction, aqua ligand substituted product, was prepared by mixing [Pt(DACH)(H₂O)₂]²⁺ and glutathione at pH 4.0 in different molar ratios viz 1:1, 1:2, 1:3 and 1:4 and thermostating the mixtures at 60°C for 48 hours. The absorption spectra of the resultant solutions were recorded and it was observed that all solutions exhibit almost identical absorbances. The composition of the product in the reaction mixture was determined by Job’s method of continuous variation. The metal-ligand ratio was found to be 1:1.

The pH of the solution was adjusted by adding NaOH/HClO₄ and the measurements were carried out with the help of a Systronics digital pH meter (model 335) with an accuracy of ±0.01 unit. Doubly distilled H₂O was used to prepare all the solutions. All other chemicals used were either of AR grade or were purified before use. During kinetic investigation, a constant ionic strength (0.1 mol dm⁻³ NaClO₄) was maintained.

Kinetic studies

Kinetic measurements were carried out on a Shimadzu spectrophotometer (UV-2101PC) equipped with a Shimadzu TB 85 thermostath (accuracy = ±0.1°C). The absorption due to glutathione was subtracted by using a 1:1 (molar ratio) ligand : water mixture in the reference cell. The progress of the reaction was monitored by following the increase in absorbance at 235 nm where the spectral difference between the reactant complex and the product
complex is 0.427. The conventional mixing technique was followed and pseudo-first order conditions with respect to the complex (1) were maintained throughout the course of the reaction. Rate data represented as an average of duplicate runs, are reproducible within ± 4%.

Results and Discussion

The pK values of glutathione are $pK_1$ (COOH) = 3.59, $pK_1$ (NH$_3$) = 8.75 and $pK_4$ (SH) = 9.65 respectively so that at pH 4.0 glutathione exists as zwitterion. The $pK_1$ and $pK_2$ for cis-diaqua(cis-1,2-diaminocyclohexane)platinum(II) have been evaluated by Irving-Rossotti titration technique and are 6.25 and 7.8 respectively. We can, therefore, assume that the substrate exists essentially as the diaqua ion.

At constant ionic strength, temperature, pH (4.0) and fixed concentration of the complex (1) (5.00 × 10$^{-5}$ mol dm$^{-3}$), the ln($D_w - D_l$) versus time ($t$) plot where $D_l$ and $D_w$ are the absorbances at time $t$ and after the completion of the reaction respectively for different ligand concentrations, is curved at the initial stage and is subsequently of constant slope. This indicates that the process of substitution follows a two step consecutive path. The first is the displacement of one aqua ligand from cis-[Pt(DACH)(H$_2$O)$_2$]$^{2+}$ (complex I) by glutathione, involving an outersphere pre-equilibrium, followed by associative interchange. The second step is the slower step, where another aqua ligand is replaced. This is the ring closure step. The anation rate constants for both the steps ($k_1$ and $k_2$) and the outer sphere association equilibrium constant ($K_E$) for the first step have been calculated according to the scheme: A → B → C , where A is the diaqua species, B is the mono substituted species and C is the final product. Formation of C from B dominates after some time has elapsed. The rate constant for the B → C step can be obtained directly from the slope of the limiting linear portion of the curve.

Calculation of $k_1$ for A → B step

The rate constant $k_{1\text{(obs)}}$ for the A → B step can be evaluated by the method of Weyh and Hamm using the usual consecutive rate law:

$$(D_w - D_l) = a_1 \exp(-k_{1\text{(obs)}} t) + a_2 \exp(-k_{2\text{(obs)}} t)$$  \hspace{1cm} (1)

whence $(D_w - D_l) - a_2 \exp(-k_{2\text{(obs)}} t)$

$$= a_1 \exp(-k_{1\text{(obs)}} t)$$  \hspace{1cm} (2)

where $a_1$ and $a_2$ are the constants dependent upon the rate constants and extinction coefficients. $a_2 \exp(-k_{2\text{(obs)}} t)$ is the value of $(D_w - D_l)$ for the second step only, hence the difference, $\Delta = a_1 \exp(-k_{1\text{(obs)}} t)$ or, $\ln \Delta = constant - k_{1\text{(obs)}} t$  \hspace{1cm} (3)

$k_{1\text{(obs)}}$ is derived from the slope of ln $\Delta$ versus time ($t$), when $t$ is small.

A similar procedure is applied for each of the following ligand concentrations: $5 \times 10^{-2}$, $6.25 \times 10^{-2}$, $7.5 \times 10^{-2}$, $1.0 \times 10^{-2}$, and $1.25 \times 10^{-2}$ mol dm$^{-3}$ at constant complex (1) concentration at pH 4.0 and at different temperatures viz, 30, 35, 40, 45 and 50°C respectively. The $k_{1\text{(obs)}}$ values thus obtained increase with the increase in ligand concentration and temperature. The $k_{1\text{(obs)}}$ values for different ligand concentrations and at different temperatures are given in Table I. The ligand concentration dependence of $k_{1\text{(obs)}}$ values can be explained in terms of rapid formation of an outersphere association complex between the reactant, complex (1) and the sulphur end of the zwitterionic form of glutathione in the A → B step. The following scheme may be proposed:

$$[\text{Pt(DACH)(H$_2$O)$_2$}]^{2+} + \text{L-LH}_2 \rightleftharpoons K_E A \text{(complex I)}$$

$$[\text{Pt(DACH)(H$_2$O)$_2$}]^{2+} \cdot \text{L-LH}_2 \rightarrow \text{outer sphere association complex}$$

$$[\text{Pt(DACH)(H$_2$O)$_2$}]^{2+} \cdot \text{L-LH}_2 \rightarrow [\text{Pt(DACH)(H$_2$O)(L-LH)}]^{+} \cdot \text{H$_2$O}^+ \rightarrow \text{cationic complex (B)}$$

where $\text{L-LH}_2$ is the zwitterionic form of glutathione. Based on the above scheme a rate expression (7) can be derived for the A → B step

$$k_{1\text{(obs)}} = k_1 K_E [\text{glutathione}] (1 + K_E [\text{glutathione}])$$  \hspace{1cm} (7)

where $k_1$ is the rate constant for conversion of outersphere complex to the inner sphere complex and...
$K_E$ is theoutersphere association equilibrium constant. Equation (9) can be rearranged as:

$$
\frac{1}{k_{\text{f(obs)}}} = \frac{1}{k_f} + \frac{1}{k_j} K_E \text{ [glutathione]} \quad \quad (8)
$$

A plot of $1/k_{\text{f(obs)}}$ versus $1/\text{[glutathione]}$ should be linear with an intercept of $1/k_j$ and slope $1/k_f K_E$. This was found to be so at all temperatures studied. The $k_j$ and $K_E$ values are obtained from the intercept and from slope to intercept ratio respectively and are included in Table 2. The observed $K_E$ values for outersphere association is higher than expected for polar groups such as $\text{NH}_3^+$, $\text{COO}^-$, $\text{SH}$, C=O etc. which affect the association constant profoundly by solvation through hydrogen bonding.

**Calculation of $k_2$ for the $B \rightarrow C$ step**

The $B \rightarrow C$ step is the ring closure step in which glutathione binds the metal centre to nitrogen with simultaneous release of the amide proton of cysteine moiety$^{15}$. This step (chelation) is however, independent of ligand concentration. At a given temperature the $k_2$ value is obtained from the slope of the $\ln([D_i-D_{i\text{lim}}]$ versus time ($t$) plot which is linear.

**Effect of temperature on reaction rate**

The reaction was studied at five different temperatures for different ligand concentrations. The anation rate constants for both the steps $A\rightarrow B$ and $B\rightarrow C$ as well as the outer sphere association equilibrium constant ($K_E$) for the first step are collected in Table 2. The activation parameters for both steps calculated from Eyring plot are given in Table 3 together with the reported values of some analogous systems.

**Mechanism and conclusion**

Glutathione (reduced form) is a tripeptide found in most living cells and is a good model compound. It is also an important biochemical reagent that might itself be a target for reaction with $[\text{Pt(DACH)}(\text{H}_2\text{O})_2]^{2+}$. The ligand exists in zwitterionic form at the experimental pH (4.0). It is well known that Pt(II), a soft acid, is more likely to form a stable bond with sulphur, a soft base, rather than with oxygen or nitrogen, both hard bases$^{12}$. Glutathione contains eight donor atoms, which gives rise to the possibility of various types of coordination$^{14}$: (i). At the N-terminal part amino acid type bond may occur; and (ii). the thiol group $\text{SH}$ may bind to the metal centre independently or may involve (S, O)

\[\begin{array}{c|c|c|c|c}
\text{Temp} (^\circ C) & 10^3 k_1 (s^{-1}) & 10^3 k_2 (s^{-1}) & K_E (dm^3 mol^{-1}) \\
\hline
30 & 2.22 \pm 0.03 & 7.78 \pm 0.01 & 531.00 \pm 0.03 \\
35 & 2.86 \pm 0.02 & 9.58 \pm 0.04 & 569.10 \pm 0.02 \\
40 & 3.64 \pm 0.03 & 11.80 \pm 0.02 & 611.10 \pm 0.04 \\
45 & 4.25 \pm 0.04 & 14.44 \pm 0.02 & 639.45 \pm 0.02 \\
50 & 5.40 \pm 0.02 & 17.52 \pm 0.02 & 678.90 \pm 0.02 \\
\end{array}\]

\[\begin{array}{c|c|c|c}
\text{Systems} & \Delta H_1 ^\circ (kJ mol^{-1}) & \Delta S_1 ^\circ (JK^{-1} mol^{-1}) & \Delta H_2 ^\circ (kJ mol^{-1}) & \Delta S_2 ^\circ (JK^{-1} mol^{-1}) & \text{Ref.} \\
\hline
\text{cis-}[\text{Pt(en)}_2(\text{H}_2\text{O})_2]^{2+} & 15.58 \pm 0.9 & -230 \pm 3.0 & 19.43 \pm 1.2 & -225.5 \pm 4.0 & 2 \\
\text{Dl-methionine} & 61.95 \pm 1.7 & -71 \pm 5.8 & 26.7 \pm 0.8 & -186.8 \pm 2.7 & 17 \\
\text{Thiourea} & 35.69 \pm 0.8 & -166 \pm 2.54 & 44.54 \pm 1.32 & -182 \pm 4.18 & 18 \\
\text{Dl-penicillamine} & 46.5 \pm 5.0 & -143 \pm 15.0 & 44.3 \pm 1.3 & -189 \pm 4.2 & 19 \\
\text{Glutathione} & 32.87 \pm 1.31 & -187.24 \pm 4.2 & 30.5 \pm 0.1 & -223.1 \pm 0.3 & \text{This work}
\end{array}\]
Scheme I—A plausible mechanism for the substitution of aqua ligands from \([\text{Pt(DACH)}(\text{H}_2\text{O})_2]^{2+}\) by glutathione.

coordination or may involve (S, N) coordination with the incorporation of deprotonated amide \(-\text{NH}-\).

Similar coordination behaviour has been proposed for palladium(II)\(^{15}\) and nickel(II)\(^{16}\) and platinum(II)\(^{13}\). In the first stage a rapid outersphere association complex formation between complex (1) and glutathione takes place and this is supported from the variation of slope in the \(k_{\text{obs}}\) versus [glutathione] plot. Job's method of continuous variation i.e. formation of 1:1 complex (product C) suggests that the 1,2-diaminocyclohexane–Pt bonds remain intact and glutathione behaves as a bidentate ligand. Thus the mechanism of the substitution of aqua ligands from cis-diaqua(cis-1,2-diaminocyclohexane)-platinum(II) ion (Scheme I) can be explained in terms of rapid formation of outersphere association complex followed by two slow consecutive steps (the first and the second steps are independent of ligand concentration). The activation parameters also suggest (Table 3) an associative mode of activation. The large negative \(\Delta S^e\) values suggest a more compact transition state i.e. associative mode of activation. For unimolecular processes (\(\Delta n = 0\)), Arrhenius activation energy term may be related to the entropy of activation of Eyring plot through the relation

\[
A = (k_B T/h) \exp(\Delta S^e/R) \exp(\Delta S^a/R) \text{ at } 25^\circ C.
\]

The frequency factor ‘A’ decreases exponentially as \(\Delta S^e\) becomes more and more negative. For the present case, \(\Delta S^e\) is more negative than \(\Delta S^a\) and ‘A’ has lower value for the second step in comparison to the first one.
In this biphasic reaction $k_2$ is slower than $k_1$, the reasons for which may be presented as follows. In the first step ($k_1$) which is the interchange step, the ‘$S$’ donor centre being a soft base got the privilege to occupy the inner-sphere position. An examination of molecular model reveals that after attachment of sulphur end of the ligand, the chelation step which involves bonding between amide group nitrogen – NH- of glutathione and platinum, takes more time to bring the vulnerable part of the ligand which is remote from the metal centre. So the low value of ‘$A$’ is expected as both parameters are interrelated.

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References