Kinetics of substitution of aqua ligands from cis-diaqua(ethylendiamine)platinum(II) perchlorate by guanosine in aqueous medium

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The kinetics of the interaction of guanosine with cis-[Pt(en)(H₂O)₂]²⁺ have been studied spectrophotometrically as a function of [Pt(en)(H₂O)₂]²⁺, guanosine and temperature at pH 4.0 where the substrate complex exists predominantly as the diaqua species. Base stacking and metal induced macrochelate formation of guanosine plays a vital role in determining the concentration limit of guanosine during kinetics. Substitution occurs in two consecutive steps; both dependent on the guanosine concentration. Activation parameters for both steps have been calculated. The low ΔHᵢ⁺ (36.28 ± 0.5 kJ mol⁻¹) and large negative values of ΔSᵢ⁺ (-125.3 ± 1.5 J K⁻¹mol⁻¹) as well as ΔHᵢ⁻ (36.63 ± 0.6 kJ mol⁻¹) and ΔSᵢ⁻ (-149.2 ± 1.9 J K⁻¹mol⁻¹) indicate associative modes of activation for both the ligand substitution processes in the two consecutive steps.

Ligand displacement reactions on square planar cis-(-N-N-)chelated platinum(II) complexes attracted continued attention due to their intrinsic chemical bonding and bio-medical applications. In particular, cis-platin, [cis–dichlorodiamineplatinum(II)] and its structural analogues are widely used in the treatment of specific cancers. It is now accepted that these platinum(II) complexes exercise their anti-tumour activity by inhibiting the replication of cellular DNA. The primary biological target appears to be the interaction with nucleic acids and therefore the binding interactions of platinum(II) amine complexes with nucleic acid and nucleic acid fragments are of great importance. Studies of numerous platinum complexes (both +4 and +2 oxidation states) reveal that anti-tumour activity requires the fulfillment of the following structural criteria:

(i) the two amine ligands must be in the cis position (for a bidentate ligand, this geometric requirement is automatically fulfilled);
(ii) the leaving group should have intermediate binding strength with respect to platinum, or should be capable of leaving easily; and
(iii) the amine ligand should have at least one N-H bond i.e. possess a hydrogen bond donor function so that it provides additional stabilisation after binding to the biological target, DNA.

Recent studies have shown that the trans-isomer also possesses anti-tumour activity. Binding interactions of -(N-N)- chelated platinum(II) complexes with mono- and oligonucleotides, nucleosides and nucleobases have been studied by NMR spectroscopy and by X-ray crystallography. Different binding modes of these nucleobases: e.g. cytosine, guanine, thymine and adenine towards platinum(II) have been suggested. Being a soft 'B' class acceptor, platinum(II) prefers nitrogen donor centers of the nucleobase sites during platination. The important binding sites are cytosine N3, guanosine N7, adenine N7 and adenine N1, among which the most preferred one appears to be the guanosine N7 site. The enhanced platinum(II) coordination of guanosine N7 has been explained in terms of hydrogen bonding. The exocyclic 6 oxo group of guanosine has been found to form hydrogen bonds with the coordinated amine ligands even in the solid state. Quantitative kinetic data for the platination processes of nucleosides and nucleotides are also available, but kinetic data for the complex formation of platinum(II) with nucleobases are rather limited.

Nucleosides and nucleotides have a considerable tendency to stack in the solution phase. This self-association has been designated as an isosmotic model of indefinite non-cooperative stacking. In the presence of metal ions, the tendency for stacking is enhanced.

In order to examine the reactivity of aqua amine complexes of platinum(II) towards guanosine in...
aqueous medium, we have undertaken the present studies.

The main purpose of this study is to investigate the role of platinum(II) to isodesmic stacking of guanosine and to evaluate the rate constants and the activation parameters.

**Materials and Methods**

Cis-[Pt(en)(H₂O)₂]²⁺ (1), prepared according to the literature method²⁹, was characterized spectroscopically.²⁰

**Determination of the analytical concentration of guanosine for macrochelate formation in the presence of cis-[Pt(en)(H₂O)₂]²⁺**

Guanosine (Fig.1) shows a considerable tendency to self-associate in aqueous solution, therefore its examination is a prerequisite for any study concerning the coordination of guanosine. Regarding the experimental conditions to be chosen for kinetic runs, one must be aware of the fact that the metal ion enhances the stacking tendencies of guanosine. For a particular metal ion concentration, the amount of product formed in solution should depend on the concentration of available monomer, guanosine. As the analytical concentration increases, the amount of product formed increases steadily, but beyond a critical analytical concentration of guanosine, the amount of product formed decreases. This result has been verified experimentally. The critical analytical concentration of guanosine for self-association and macrochelate formation in the presence of platinum(II) ions has been determined by UV-vis spectroscopy.

The product of the reaction between cis-[Pt(en)(H₂O)₂]²⁺ and guanosine shows characteristic peaks at 217 nm and 275 nm. As the kinetics of the reaction were monitored by observing the increase in absorbances at 221 nm, it is assumed that the peak height (H) at 221 nm is a function of the amount of product formed. The product of the reaction between cis-[Pt(en)(H₂O)₂]²⁺ (1) and guanosine was obtained by mixing the two reactants at pH 4.0 and thermostating the mixture at 60°C for 48 h. For a fixed metal ion concentration, the peak height (H) at 221 nm was plotted against the analytical concentration of guanosine (Fig. 2). It shows a maxima at 0.6 mM guanosine concentration. This is the critical analytical concentration of guanosine beyond which macrochelate formation is predominant in the presence of the platinum(II) ion.

**Evaluation of rate constants and activation parameters**

The kinetics of the reaction between cis-[Pt(en)(H₂O)₂]²⁺ and guanosine were monitored at constant complex (1) concentration (0.00002 mol dm⁻³) under pseudo-first order conditions so that the guanosine concentration did not exceed 0.0006 mol dm⁻³. Otherwise macrochelate formation will hinder the reaction kinetics. The product (bis-substituted complex) of the reaction was prepared by mixing the two reactants at pH 4.0 in different molar ratios: viz. 1:2, 1:3, 1:4 and thermostating the mixtures at 60°C for 48 h. All three solutions so prepared exhibited almost identical absorbances at 221 nm. 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 1:2. The spectral difference between the product complex and the substrate complex is shown in Fig. 3.

The time scan spectra in the reaction of cis-[Pt(en)(H₂O)₂]²⁺ with guanosine under pseudo-first order condition with respect to guanosine in the range 200-300 nm shows that two consecutive stages take place which is characterised by isosbestic points at

![Fig. 1.—The syn- and anti-conformation of guanosine.](image)

**Fig. 2.—Peak height (H) at 221 nm versus analytical concentration of guanosine; A: with constant [complex (1)] =2.0 × 10⁻⁵ mol dm⁻³; B: with constant [complex (1)] = 3.0 × 10⁻⁵ mol dm⁻³.**
consistent with the isodesmic model of indefinite non-cooperative stacking. The association can be written

$$A_n + A_{sl} \rightarrow A_{sl}; K = \frac{[A_{sl}][A]}{[A_n]}$$

where $K$ is the association constant ($K = 8.0 \pm 3 M^{-1}$).

Self-association of guanosine proceeds beyond the dimer, the distance between the stacked molecules being of the order of 0.35 nm. In an earlier experiment, 25 mM guanosine was used to avoid excessive stacking. In this paper, we have examined the critical analytical concentration of guanosine by UV-vis spectroscopy in the presence of the platinum(II) ion beyond which monomeric guanosine is not available in solution. The critical analytical concentration was found to be 0.6 mM (Fig. 2). Hence, to be on the safe side for monomeric guanosine, all kinetic runs were carried out in solutions with $[guanosine] \leq 0.5$ mM. The ring current in the base moiety of nucleosides and nucleotides is responsible for such self-stacking. A comparison of the equilibrium constant shows a decreasing tendency in the series adenosine > guanosine > hypoxanthine > cytosine ~ uracil. Metal ion enhances the stacking of guanosine by the formation of macrochelate.

The $pK_1$, $pK_2$, and $pK_3$ values for guanosine are 2.14, 9.03 and 12.3 which refer to the dissociation of guanosine monocation, base N1H and sugar chain (5') respectively.

The $pK_1$ and $pK_2$ for cis-diaqua(ethylene-diamine)platinum(II) are 5.8 and 7.6 respectively, and we can assume that at pH 4.0 the reactant exists as the diaqua ion. At constant temperature, constant pH 4.0 and at a fixed concentration of complex (I), the $\ln(D_2 - D_1)$ versus time ($t$) plot, where $D_1$ and $D_2$ are the absorbances at time $t$ and after the completion of the reaction respectively, is curved at the initial stage and is subsequently of constant slope. This indicates that the reaction is not a single step process: a two step consecutive process is assumed, both steps being dependent on ligand concentration. In the first step one aqua ligand is displaced from cis- \[\text{Pt(en)(H}_2\text{O)}_2\text{]}^{2+}, \text{complex (I)} \text{ by guanosine. The second-step is slower and another aqua ligand is replaced by guanosine. Steric retardation leads to a decrease in the rate.}

The reaction rate constants for both steps ($k_1$ and $k_2$) have been evaluated according to the following:

$$k_{1(\text{obs})} \quad k_{2(\text{obs})}$$

$$A \rightarrow B \rightarrow C$$
where A is the diaqua species (complex 1), B is the single substituted species, and C is the final product (complex 2); $[\text{Pt(en)}(\text{Gua})_2]^{2+}$. Formation of C from B predominates after some time has elapsed.

**Calculation of $k_1$ for the $A \rightarrow B$ step**

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

$$(D_\infty - D_i) = a_1 \exp(-k_{\text{obs,1}}t) + a_2 \exp(-k_{\text{obs,2}}t) \quad \ldots (1)$$

where $a_1$ and $a_2$ are constants depending upon the rate constants and extinction coefficients. Rearranging Eq. (1),

$$(D_\infty - D_i) - a_2 \exp(-k_{\text{obs,2}}t) = a_1 \exp(-k_{\text{obs,1}}t)$$

Values of $(D_\infty - D_i) - a_2 \exp(-k_{\text{obs,2}}t)$ are obtained from the difference between X and Y i.e. $(X - Y)$ in the plot of $\ln{(D_\infty - D_i)}$ versus time (t) curve (Fig. 4) so that $\Delta = a_1 \exp(-k_{\text{obs,1}}t)$ and putting $\ln{\Delta} = \text{constant} - k_{\text{obs,1}}t$, we obtain $\Delta$ by extrapolation. Then $k_{\text{obs,1}}$ is derived from the slope of $\ln{\Delta}$ versus time (t) (Fig. 5) when $t$ is small. The rate constant $k_{\text{obs,2}}$ can be obtained directly from the slope of the linear portion of the $\ln{(D_\infty - D_i)}$ versus time (t) curve. The $k_{\text{obs,1}}$ values for different ligand concentrations at different temperatures are given in Table 1.

A similar procedure is applied to each ligand (guanosine) concentration in the 0.0002-0.0005 mol dm$^{-3}$ range at constant complex (1) concentration (0.00002 mol dm$^{-3}$) at pH 4.0 and at 40, 45, 50, 55 and 60°C respectively. Kinetic runs could not be performed outside this concentration range because of self-association and metal assisted macrochelate formation. The $k_{\text{obs,1}}$ values obtained are linearly dependent on [guanosine].

The following scheme (Scheme 1) is proposed.

$$
\begin{align*}
\text{[Pt(en)(H}_2\text{O})_2]^{2+} + \text{Gua} & \rightarrow \text{[Pt(en)(H}_2\text{O})(\text{Gua})]\text{[H}]^{2+} + \text{H}_2\text{O} \quad \ldots (2) \\
\text{[Pt(en)(H}_2\text{O})(\text{Gua})]\text{[H]}^{2+} + \text{Gua} & \rightarrow \text{[Pt(en)(Gua)]}_2^{2+} + \text{H}_2\text{O} \quad \ldots (3)
\end{align*}
$$

**Scheme 1**

Based on the above Scheme 1 above, a rate expression can be derived for the $A \rightarrow B$ step

$$\frac{dB}{dt} = k_1 [\text{Pt(en)(H}_2\text{O})_2]^{2+}\text{[Gua]} \quad \ldots (4)$$

where $[\text{Pt(en)(H}_2\text{O})_2]^{2+}$ is the concentration of the unreacted complex and [Gua] is the concentration of guanosine.

**Table 1**

<table>
<thead>
<tr>
<th>$[\text{Gua}]$ (mol dm$^{-3}$)</th>
<th>40°C</th>
<th>45°C</th>
<th>50°C</th>
<th>55°C</th>
<th>60°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.0</td>
<td>3.28</td>
<td>4.11</td>
<td>5.20</td>
<td>6.46</td>
<td>8.01</td>
</tr>
<tr>
<td>3.0</td>
<td>4.92</td>
<td>6.18</td>
<td>7.79</td>
<td>9.69</td>
<td>12.04</td>
</tr>
<tr>
<td>3.5</td>
<td>5.75</td>
<td>7.20</td>
<td>9.09</td>
<td>11.31</td>
<td>14.04</td>
</tr>
<tr>
<td>4.0</td>
<td>6.56</td>
<td>8.23</td>
<td>10.39</td>
<td>12.91</td>
<td>16.03</td>
</tr>
<tr>
<td>5.0</td>
<td>8.22</td>
<td>10.28</td>
<td>12.99</td>
<td>16.14</td>
<td>20.07</td>
</tr>
</tbody>
</table>

Fig. 5—A typical kinetic plot of $\ln{\Delta}$ versus time t. [complex (1)] = 2.0 x 10$^{-5}$ mol dm$^{-3}$; [guanosine] = 4.0 x 10$^{-4}$ mol dm$^{-3}$, temp = 50°C
Hence we can write, \( k_{2\text{obs}} = k_2[G\text{ua}] \), where \( k_2 \) is the second order rate constant for the first aqua ligand substitution. A plot of \( k_{2\text{obs}} (s^{-1}) \) versus [guanosine] (mol dm\(^{-3}\)) should be linear, passing through the origin. This was found to be so at all temperatures studied. The rate constant \( k_2 \) values, calculated from the slopes of the \( k_{2\text{obs}}(s^{-1}) \) versus [guanosine] (mol dm\(^{-3}\)) plot at different temperatures, are collected in Table 2.

**Calculation of \( k_2 \) for the \( B \rightarrow C \) step**

The second step is the interaction of another guanosine molecule with cis-[Pt(en)(H\(_2\)O)(Gua)]\(^2+\) where the second aqua molecule has been substituted. At a particular temperature the \( k_{2\text{obs}} \) values calculated from the limiting linear portion of ln \((D_x-D_y)\) versus time \((t)\) curve (when \( t \) is large) were found to be linearly dependent on [guanosine] (Table 3). The \( k_2 \) values (the anation rate constant for the second step) were obtained from the linear plot of \( k_{2\text{obs}}(s^{-1}) \) versus [guanosine] (mol dm\(^{-3}\)) and are given in Table 2.

**Effect of temperature on reaction rate**

The reaction was studied at five different temperatures for different ligand concentrations. The temperature dependence results in the activation parameters (calculated from Eyring plots) quoted in Table 4 and has been compared with those for analogous systems.

**Mechanism and conclusion**

The present investigation of aqua ligand substitution from cis-diaqua(ethylenediamine)-platinum(II) ion by guanosine shows two consecutive processes, both dependent on guanosine concentration.

The activation parameters \( (\Delta H_1^a = 36.28 \pm 0.5 \text{ kJ mol}^{-1}, \Delta S_1^a = -125.3 \pm 1.5 \text{ J mol}^{-1}) \) for the first step and second step \( (\Delta H_2^a = 36.03 \pm 0.6 \text{ kJ mol}^{-1}, \Delta S_2^a = -149.2 \pm 1.9 \text{ J mol}^{-1}) \) suggest an associative mode of activation for the substitution processes. The low \( \Delta H_1^a \) and \( \Delta H_2^a \) values imply a good degree of ligand participation in the transition state. The enhanced kinetic lability of guanosine N7 can be explained by the formation of hydrogen bond between the exocyclic 6 oxo group and the N-H of the diamine residue bound to platinum(II) centre. The second step is the slower one in which another guanosine interacts with the mono aqua substituted platinum(II) species (B). The possible structures of mono ligand species (B) are shown in Table 4 and has been compared with those for analogous systems.

**Table 2—\( k_1 \) and \( k_2 \) values for the substitution reaction**

<table>
<thead>
<tr>
<th>Temp (°C)</th>
<th>( k_1 ) (dm(^{-1}) mol(^{-1}) s(^{-1}))</th>
<th>( 10^4 k_2 ) (dm(^{-1}) mol(^{-1}) s(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>1.64 ± 0.003</td>
<td>10.1 ± 0.01</td>
</tr>
<tr>
<td>45</td>
<td>2.04 ± 0.003</td>
<td>12.9 ± 0.02</td>
</tr>
<tr>
<td>50</td>
<td>2.61 ± 0.002</td>
<td>16.4 ± 0.01</td>
</tr>
<tr>
<td>55</td>
<td>3.22 ± 0.003</td>
<td>20.1 ± 0.01</td>
</tr>
<tr>
<td>60</td>
<td>4.01 ± 0.005</td>
<td>24.7 ± 0.01</td>
</tr>
</tbody>
</table>

**Table 3—10\(^4\) \( k_{2\text{obs}}(s^{-1}) \) values for different [guanosine] at different temperatures**

<table>
<thead>
<tr>
<th>[Gua] (mol dm(^{-3}))</th>
<th>( 10^4 k_{2\text{obs}}(s^{-1}) ) at temp =</th>
</tr>
</thead>
<tbody>
<tr>
<td>40°</td>
<td>2.04, 2.54, 3.26, 4.01, 4.96</td>
</tr>
<tr>
<td>45°</td>
<td>3.05, 3.81, 4.88, 6.01, 7.45</td>
</tr>
<tr>
<td>50°</td>
<td>3.57, 4.44, 5.70, 7.02, 8.69</td>
</tr>
<tr>
<td>55°</td>
<td>4.09, 5.08, 6.51, 8.03, 9.91</td>
</tr>
<tr>
<td>60°</td>
<td>5.11, 6.34, 8.16, 10.01, 12.41</td>
</tr>
</tbody>
</table>

**Table 4—Activation parameters for analogous systems**

<table>
<thead>
<tr>
<th>Systems</th>
<th>( \Delta H_1^a ) (kJ mol(^{-1}))</th>
<th>( \Delta S_1^a ) (J K(^{-1}) mol(^{-1}))</th>
<th>( \Delta H_2^a ) (kJ mol(^{-1}))</th>
<th>( \Delta S_2^a ) (J K(^{-1}) mol(^{-1}))</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>cis-<a href="H(_2)O">Pt(NH(_3)</a>(^2+)]</td>
<td>31.2 ± 4.3</td>
<td>-117 ± 15</td>
<td>60.8 ± 5.3</td>
<td>-55 ± 17.9</td>
<td>35</td>
</tr>
<tr>
<td>S'-dG MPH</td>
<td>40.64 ± 4.4</td>
<td>-106 ± 16</td>
<td>62.8 ± 1.5</td>
<td>-46.3 ± 5.2</td>
<td>31</td>
</tr>
<tr>
<td>S'-GMPH</td>
<td>35.6 ± 3.0</td>
<td>-166.0±8.0</td>
<td>26.8 ± 2.0</td>
<td>-195.0 ± 4.0</td>
<td>48</td>
</tr>
<tr>
<td>ciss-[Pt(en)(H(_2)O)(^2+)]</td>
<td>61.95 ± 1.7</td>
<td>-71 ± 5.8</td>
<td>26.7 ± 0.8</td>
<td>-186.8 ± 2.7</td>
<td>49</td>
</tr>
<tr>
<td>Thiourea</td>
<td>43.16±1.44</td>
<td>-122.9±2.5</td>
<td>39.61±0.8</td>
<td>-203.8±1.2</td>
<td>52</td>
</tr>
<tr>
<td>DL-penicillamine</td>
<td>46.5±4.6</td>
<td>-143.0±15.0</td>
<td>44.3±1.3</td>
<td>-189.0±4.2</td>
<td>50</td>
</tr>
<tr>
<td>5'-AMP</td>
<td>42.76±1.64</td>
<td>-112.1±5.1</td>
<td>58.1±1.4</td>
<td>-84.2±4.4</td>
<td>51</td>
</tr>
<tr>
<td>Guanosine</td>
<td>36.28±0.5</td>
<td>-125.3±0.5</td>
<td>36.03±0.6</td>
<td>-149.2±1.9</td>
<td>this work</td>
</tr>
</tbody>
</table>

[Ref. APRIL 2002]
substituted (B) and the bis-substituted final product (C) (Fig. 6a and 6b respectively) possess hydrogen bonds leading to a more ordered structure compared to the reactant complex. This is reflected in the negative entropy of activation for both the steps.

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References


Fig. 6—The possible hydrogen bonded structure of (a) mono-substituted complex (B); (b) bis-substituted final product (C)

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