Coenzyme B<sub>12</sub> model studies: Electronic and steric \textit{trans} effects of cobaloximes

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The equilibrium, kinetics and thermodynamic parameters of axial ligand substitution of cobaloximes by -CONH<sub>2</sub> and -CSNH<sub>2</sub> containing molecules in three different cobaloximes viz., \textit{trans} RCo(DH)H<sub>2</sub>O where DH = mono anion of dimethyl glyoxime and R = CH₃, CH<sub>2</sub>I and CH₂Br have been studied as a function of pH, incoming ligand concentration and temperature. It has been found that the incoming ligand substitutes the coordinated water molecule \textit{trans} to the alkyl group with equilibrium constants that follow the order of stability: CH₂Br > CH₂I > CH₃. Rate and activation parameters enable the formulation of the reaction mechanism that can account for the substitution reaction of the investigated cobaloximes. The stability of the alkyl (aq) cobaloximes has been explained on the basis of pK<sub>a</sub>, basicity and steric influence of the incoming ligand. Alkyl cobaloximes are interesting compounds not only due to their analogies with organo cobalamins but also for their extensive coordination chemistry. For these complexes the kinetics of substitution of the axial ligand L has been extensively studied. It has been shown that the nature of the alkyl group (R) significantly affects the substitution rate of the labile \textit{trans} group, i.e., as the electron donating ability of the R group increases the rate constant also increases.

It has been concluded that in non-coordinating solvents the ligand substitution reaction occurs via a limiting dissociative (D) mechanism, which indicates the presence of a five-coordinate intermediate. However, in coordinating solvents the suggested mechanism is the object of controversial opinions. Hamza et al. suggested that the ligand substitution reaction mechanism in the case of alkyl(aquo)cobaloximes involves the five-coordinate RCo(DH)₂ complex in cobaloximes (where R = cyclo-C₅H₅(c-P), CH₃CH₂ (Et), CH₃= (Me), C₆H₅CH₂ (Bz), C₆H₅ (Ph) and CF₃CH₂). They also suggested that in various alkyl cobalamins (where R = CN, CNCH₃, CF₃, CF₃CH₂, CF₃H, CH₃ and BrCH₂) and cyanomimidazolyl cobamide the operating mechanism is dissociative, and also showed that the nature of alkyl group (steric effect) has an influence on the kinetics and mechanistic aspects of the axial ligand substitution reactions in cobaloximes.

Satyanarayana et al. have reported the substituent effect of R on Co-C bond, when the substituent changes from an electron donating (σ donor CH₃) to electron withdrawing group (CH₂Br, CH₂I). Reasonable hypothesis about the nature of the transition state could be formulated by utilizing the thermal activation parameters ΔH<sup>e</sup> and ΔS<sup>e</sup>. These parameters have been determined for a series of substitution reactions as function of R<sup>15-17</sup>.

Experimental

All solvents, reagents and other organic chemicals were either obtained as pure from commercial sources or were purified by distillation in the usual manner. The alkyl(aquo)cobaloximes were prepared by the procedure of Brown et al. All manipulations were performed under minimal illuminations due to photolability of organo cobalt bond. K<sub>app</sub> values, for the axial ligation of alkyl(aquo)cobaloximes were determined by spectrophotometric measurements at the single wavelength of the corresponding cobaloxime, as a function of pH. Elico single beam spectrophotometer (SL 171 model) with the sample compartment thermostatted at 25 ± 0.1°C was used for spectrophotometric measurement.

In a 3ml cuvette, RCo(DH)H<sub>2</sub>O, an appropriate buffer (0.2 M) to maintain pH, KCl to maintain ionic strength 1.0 M and varying concentrations of ligand (6×10<sup>−3</sup>M–6×10<sup>−2</sup> M) were taken in a cell block maintained at 25 ± 0.1°C. Solutions were allowed to temperature-equilibrate in the spectrophotometer cell block for at least 15 minutes prior to addition of cobaloxime. Final absorbance readings were taken after equilibrium was established as indicated by the time independence of the readings. The data were analyzed by least squares fit to Eq. 1.

\[ \Delta A = \Delta A_{max} - 1/K_{app} \Delta A/[L]_i \]  

\[ \Delta A \text{ plotted as a function of } \Delta A / [L]_i \text{ has the slope } -1 / K_{app}, \text{ where } \Delta A \text{ is the difference in absorbance between solutions containing cobaloxime (6×10<sup>−3</sup>M) and added ligand (L) (6×10<sup>−3</sup>M–6×10<sup>−2</sup>M) and solutions containing only cobaloxime at the same} \]
concentration. The least-squares fit of the data (performed with the aid of computer program using MS-Excel 97 package) yields \( K_{\text{app}} \).

Kinetic studies were carried out for binding of ligands to bromomethyl(aquo)cobaloxime and iodomethyl(aquo)cobaloxime. This was not possible for methyl(aquo)cobaloxime by conventional spectrophotometry, as the reactions was very fast. For each ligand, \( L \), at varying \( \text{pH} \), first order rate constant \( k_{\text{obs}} \) were determined from the absorbance measurements at the same wavelength used for \( K_{\text{app}} \) determinations under pseudo-first order condition with \( L \).

Reaction progress was monitored by measurement of the change in the absorbance upon addition of cobaloxime to a 3 ml cuvette containing KCI to maintain unit ionic strength, necessary buffer (0.2 \( M \)) to maintain \( \text{pH} \) and ligand in the thermostated (25 ± 0.1°C) cell compartment.

Kinetic traces were monitored for at least five half times and analyzed by plotting \( \ln A_t - \ln A_0 \) versus \( t \) using standard linear regression analysis\(^\text{\textsuperscript{20}}\) to obtain a pseudo-first order rate constant, \( k_{\text{obs}} \). Second-order rate constants, \( k_{\text{on}} \) at a given \( \text{pH} \) for a given ligand were obtained from the slopes of least-squares fits of the data \( k_{\text{obs}} \) versus ligand concentration while \( k_{\text{off}} \) is the time dependent dissociation rate constant.

\[
k_{\text{obs}} = k_{\text{on}} [L]_t + k_{\text{off}} \quad \ldots \quad 2
\]

The rate constant is sensitive to changes of temperature. The activation parameters (\( \Delta H^\ddagger \) and \( \Delta S^\ddagger \)) were calculated from an Eyring plot.

**Results and discussion**

The equilibrium, kinetic and thermodynamic parameters of the axial ligand substitutions in the cobaloximes (a model for coenzyme \( \text{B}_{12} \)) is a subject of interest due perhaps to their influence in the coenzyme \( \text{B}_{12} \) enzymatic processes. The elusive mechanism of \( \text{B}_{12} \) dependent enzymatic process involves the Co-C bond homolysis\(^\text{\textsuperscript{21}}\), which is influenced by both equatorial (cis influence) and trans ligand (trans influence) in coenzyme \( \text{B}_{12} \).

Recently Sudershan Reddy et al.\(^\text{\textsuperscript{22}}\) studied the axial ligation reactions of urea and thiourea with benzyl(aquo)cobaloxime to show that soft ‘S’ donor (thiourea) forms more stable complex than hard O donor urea with soft Co(III) centre. Co (III) is assigned as soft acid character\(^\text{\textsuperscript{23}}\). Further, vitamin \( \text{B}_{12} \), methyl(aquo)cobalamin and methyl(aquo)cobaloxime (Str I) are assigned as soft or class “b” character.

Figure 1 shows the binding of \( \text{BrCH}_2\text{Co(DH)}_2\text{OH}_2 \) with varying concentration of ligand and indicates that as the concentration of ligand increases the absorbance decreases at \( \lambda_{\text{max}} \). The order of stability with respect to amides and thiouamides is that ‘S’ donor ligands have greater stability than the corresponding ‘O’ donor ligand and is based on HSAB principle. The order of stability among amides and thiouamides (Table I) is: AC < SC < TAC < U < FA < TU < TSC.

The ‘S’ end of the thiouamide is a soft donor and has greater affinity for the soft metal centre cobalt (III). Hence, the thiouamides have greater stability constant than the corresponding amide. Steric crowd on the ligand also plays an important role in the

![Fig. 1—Dependence of the binding of BrCH2Co(DH)2OH2 with varying concentration of ligand. [Isosbestic point =420.4 nm]](image)
Table 1—Comparative formation constants for the axial ligation of alkyl(aquo) cobaloximes $\text{RCO(DH)}_2\text{OH}$ by different ligands (L) at 25°C in aqueous solution, ionic strength 1.0 M (KCI)

<table>
<thead>
<tr>
<th>Ligand(L)</th>
<th>$pK_a$</th>
<th>$K_{eq}(M^{-1})$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea</td>
<td>0.10</td>
<td>102.38</td>
</tr>
<tr>
<td>Formamide</td>
<td>0.12</td>
<td>108.11</td>
</tr>
<tr>
<td>Thiourea</td>
<td>0.30</td>
<td>118.87</td>
</tr>
<tr>
<td>Acetamide</td>
<td>0.63</td>
<td>6.7150</td>
</tr>
<tr>
<td>Thioacetamide</td>
<td>1.68</td>
<td>74.61</td>
</tr>
<tr>
<td>Thiosemicarbazide</td>
<td>1.88±0.2</td>
<td>508.70</td>
</tr>
<tr>
<td>Semicarbazide</td>
<td>3.3±0.2</td>
<td>64.61</td>
</tr>
</tbody>
</table>

stability of the complex (Fig. 2). Hence, formamide forms more stable complex than acetamide, even though acetamide is more basic than formamide.

The kinetics of ligand addition to complex are strongly dependent upon pH, since only the deprotonated ligand will coordinate to Co (III). All ligand (Scheme 1) substitution reactions of $\text{B}_{12}$ and alkyl(aquo)cobaloximes reported to date are second order with rate-limiting addition of L. Figure 3 shows the dependence of pseudo-first order rate constant for association as a function of pH.

The plots of $k_{obs}$ versus concentrations of ligand are linear. From the slopes of $k_{obs}$ versus concentration of [L], second order rate constant $k_{on}$ have been calculated. The pH independent second order rate constant $k_{on}$ is calculated as $k_{on} = k_{eq}/[\alpha]$, where, $\alpha$ is the degree of dissociation of the ligand at a given pH.

The pH independent second order rate constant follows the order of basicity of the ligands (Table 2).

The small difference in the rate of ligand substitution despite large differences in the stabilities of their coordination complexes for the usual Co (III) complexes and aquo cobalamin have been taken to indicate a lack of significant activation of the transition state by the incoming ligand. Conversely, domination of the transition state activation by the leaving ligand indicates dissociative interchange mechanism ($I_d$). In this, the incoming ligand and leaving ligand are loosely bound to cobalt in the transition state and may be indicated as L--Co--HOH.

A study on the substituent effect of R on Co-C bond by Satyanarayan25 reveals that when the substituent changes from an electron donating group (R = CH$_3$, a $\sigma$ donor) to electron withdrawing groups (R=CH$_2$Br, CH$_2$I, . . .) the electron density on the cobalt decreases and Co-OH$_2$ bond becomes more inert to substitution. This strongly suggests that delocalization is responsible for the resonance effect which stabilizes the positive charge on the metal making it more electrophilic as shown below.

$$\delta^- \text{Br}^- \text{CH}_2 \delta^+ \text{Co(DH)}_2 \text{L}$$

The effect of systematically changing the alkyl group in trans- $\text{RCO(DH)}_2\text{H}_2\text{O}$ was investigated. It has
been conclusively proved that, in non-coordinating solvents the ligand substitution reaction occurs via a limiting dissociative (D) mechanism, which implies the presence of a five coordinate intermediate. The mechanism may be depicted as:

\[
\begin{align*}
\text{RCOL} & \quad \leftrightarrow \quad \text{RCO} + L \quad k_1 k_{-1} \\
\text{RCO} + L' & \quad \rightarrow \quad \text{RCOL'} \quad k_2
\end{align*}
\]

It has been shown that the nature of the alkyl group (R) significantly affects the substitution of the labile trans group, i.e., as the electron donating ability of the R group changes the rate constant also changes. By way of comparison, the lability of trans RCo(DH)\textsubscript{2}H\textsubscript{2}O is affected by the steric and electronic effects of the alkyl group (R). Reenstra and Jeneckes\textsuperscript{32} pointed out that even a small amount of nucleophilic participation of the incoming ligand in the transition state would result in a significant rate increase if the intimate mechanism of the reaction is I.d.

The above experiments indicate that activation enthalpy increases as the electron withdrawing ability of R increases. Table 3 shows that the change in rate is caused by random changes in both \(\Delta H^\ddagger\) and \(\Delta S^\ddagger\), so that a linear correlation between \(\Delta H^\ddagger\) and \(\Delta S^\ddagger\) (isokinetic relationship) could not be observed. This suggests that these parameters are influenced by a combination of both steric and electronic effects, which differ for each R groups. The contributions of the steric and electronic effects of the alkyl groups cannot be separated.

The \(\Delta H^\ddagger\) values for the substitution reactions of trans \([\text{RCO(DH)H}_2\text{O}]\) for R= PhCH\textsubscript{2} and CF\textsubscript{3}CH\textsubscript{2} by pyridine derivatives in H\textsubscript{2}O and methanol are relatively independent of the nature of the incoming ligand. It is expected that the reactions involve a dissociative activation process, since the ligands are loosely bound in the transition state. The activation enthalpy (\(\Delta H^\ddagger\)) for R = CF\textsubscript{3}CH\textsubscript{2} is higher than that for R = PhCH\textsubscript{2} due to strong Co-OH\textsubscript{2} bond in the ground state. Hence, \(\Delta H^\ddagger\) values appear to be affected by both electronic and steric effects of the R group\textsuperscript{33}.

The above study shows that kinetic and activation parameters are influenced by a combination of both steric and electronic effects which differ for each R groups. The contributions of the steric and electronic effects of the alkyl groups cannot be separated.