Kinetics and equilibria of ligand substitution reactions of bromomethyl(aquo)cobaloxime with substituted imidazoles

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Equilibrium constants and rate of formation and dissociation for the binding of L to BrCH₂Co(DH)₂OH₂ (where L = imidazole, 1-methylimidazole, 2-methylimidazole, 1,2-dimethylimidazole and 2-ethylimidazole) have been determined spectrophotometrically. The binding constants and rate of formation increase in the order 1-Me IMD > IMD > 2-Me IMD > 1,2-DM IMD > 2-Et IMD. The data has been interpreted based on the basicity of the ligand, steric hindrance and π back bonding from Co(III)→L and hard and soft interactions.

Organobis(dimethylglyoximato)(aquoa) cobalt(III) compounds, trivially known as organo cobaloximes are accepted as coenzyme B₁₂ model compounds. The study of such complexes unfolds the mechanism of B₁₂ dependent enzymatic reactions especially at the initial stages of the B₁₂ catalytic cycle. In addition to this, B₁₂ model compounds paved the way for exciting organometallic chemistry arising out of homolytic and heterolytic cleavage of the cobalt – carbon bond and make an impact on modern methodology of synthesis.

For B₁₂ model studies, alkyl(aquo)cobaloximes are preferred to pyridine or other Lewis base donor complexes for several reasons. First, the alkyl(aquo)cobaloximes are considerably more soluble in water and water-alcohol mixtures than pyridine complexes.

The kinetics and equilibria of the reaction of alkyl(aquo)cobaloxime with imidazole and substituted imidazoles were studied as a function of pH at 25°C at ionic strength 1.0 mol dm⁻³ (maintained by KCl) spectrophotometrically. The rate of substitution of water varies linearly with the $pK_a$ of the incoming ligand. Imidazoles were chosen as the imidazole rings were present in nucleic acids, proteins, antibiotics, and cofactors apart from being present in biological systems.

Materials and Methods

Imidazole, 1-methylimidazole, 2-methylimidazole, 2-ethylimidazole and 1,2-dimethylimidazole, were obtained in highest purity from Acros. Potassium chloride, HPLC grade, methanol, acetic acid, hydrochloric acid, phosphoric acid and formic acid were obtained from fluka. Doubly distilled, deionized water was used throughout the study. pH was maintained by employing 0.2 M acetate, phosphate or tris-HCl buffers depending on the pH.

Alkyl(aquo)cobaloximes were prepared by the procedure of Brown, et al. as follows:

\[
\text{Co(OAc)}_2 4\text{H}_2\text{O} + 2(\text{DH})_2 \rightarrow \frac{1}{2} [\text{Co}^{11}(\text{DH})_2\text{OH}_2]^2 + 2\text{ACOH} + 4\text{H}_2\text{O}
\]

\[
\frac{1}{2}[\text{Co}^{11}(\text{DH})_2\text{OH}_2]^2 + \frac{1}{2}\text{H}^+ + \frac{1}{2}\text{H}^\rightarrow \text{HCo(DH)}_2\text{OH}_2
\]

\[
\text{HCo(DH)}_2\text{OH}_2 + \text{RI} \rightarrow \text{HCo(DH)}_2\text{OH}_2 + \text{HI}
\]

All manipulations were done under minimal illuminations as the organo cobalt bond is photolabile. 200 ml of methanol is deaerated under a stream of nitrogen for 30 min. in a three-necked round-bottom flask fitted with a pressure equalizing funnel. Cobaltous acetate tetrahydrate (0.05 mole) and dimethyl glyoximine (0.1 mole) were added and stirred magnetically under a continuous N₂ purge. The mixture was stirred for 30 min. to ensure complete formation of [Co(DH)₂OH₂]. Potassium hydroxide (0.102 mole) in deaerated water was added to neutralize the acetic acid and then the reaction mixture was ice cooled. Dibromo methane (0.055 mole) was added into mixture for the alkylation followed by the addition of 1.2 g sodium borohydride in 40 ml deaerated water in which 0.1 g KOH was
already dissolved was added drop wise, slowly over a period of about one hour. Formation of the bright yellow orange product was monitored by TLC, whereas unalkylated cobaloximes remained at the origin. When product formation was virtually completed by (TLC assay) the reaction mixture was filtered (suction) and then reduced to 100 ml employing rotary evaporator, diluted with 100 ml water and allowed to crystallize overnight at 4°C. Suction filtration, air drying, and drying in vacuo over CaCl$_2$ provided alkyl(aquo)cobaloximes (80%) of red powder. The solid was reprecipitated from a concentrated methanolic solution by addition of water and cooling on ice. The above compounds were confirmed by $^1$H NMR in DMSO-d$_6$.

These alkyl(aquo)cobaloximes are photolabile, particularly in solution. Soluble in alcohol and DMSO, less so in chloroform or water and virtually insoluble in ether and hydrocarbon solvents.

All work with the alkyl(aquo)cobaloximes was performed in dim light (in dark room) and solutions were covered with aluminum foil. pH measurements were made with a Digisun digital pH meter equipped with a combined glass electrode. The electrode was standardized at two pH values (pH = 4 and 9.2) with standard buffer solutions.

UV-visible spectra were recorded on a Hitachi U-3410 keeping the sample in a thermostated cell holder. Single wavelength measurements were made on Elico single beam spectrophotometer SL 171 model, the sample compartment of which was thermostated at 25 ± 0.1°C.

**Equilibrium measurements**

Apparent equilibrium constants ($K_{app}$) for the axial ligation of alkyl(aquo)cobaloximes (Scheme 1) were determined by spectrophotometric measurements at the $\lambda_{max}$ 436 nm of the BrCH$_2$Co(DH)$_2$OH$_2$. In 3ml cuvette, solutions containing BrCH$_2$Co(DH)$_2$OH$_2$, an appropriate buffer (0.2 M) to maintain pH, and KCl to maintain ionic strength at 1.0 M and varying concentrations of ligand were taken and allowed to equilibrate in a thermostated cell holder at 25 ± 0.1°C for atleast 15min. prior to addition of cobaloxime.

$$K_{app} = \frac{[RCo(DH)_2L][RCo(DH)_2H_2O][L]_{free}}{[RCo(DH)_2H_2O+L]}$$ \hspace{1cm} \ldots (4)

$$K_{app} = \frac{k_{on}}{k_{off}}$$

**Scheme 1**

Final absorbance readings were taken after equilibrium was established as indicated by the time independence of the readings. For such experimental setups, at a given pH, Eq. (6) is applied

$$\Delta A = \frac{\Delta A_{max}}{[L]_f} \left( \frac{1}{K_{app} + [L]_f} \right)$$ \hspace{1cm} \ldots (6)

where $\Delta A$ is the difference in absorbance between solutions containing cobaloxime and added ligand (L) and solutions containing only cobaloxime at the same concentration, $\Delta A_{max}$ is the maximum absorbance change thus obtained at high [L], and [L]$_f$ is the equilibrium concentration of the ligand in both ionization states. The data were analyzed by a least squares fit to the rearranged form of Eq.(6) to give Eq.(7)

$$\Delta A = \frac{\Delta A_{max} - 1}{K_{app}[L]_f} \left( \frac{1}{K_{app} + [L]_f} \right)$$ \hspace{1cm} \ldots (7)

$$[L]_f = [L]_{tot} - C_T \Delta A / \Delta A_{max} \ldots \ldots (8)$$

$[L]_f$ is calculated from Eq.(8) using measured value of $\Delta A_{max}$, where $[L]_{tot}$ is the total concentration of added ligand and $C_T$ is the total concentration of cobaloxime. $\Delta A$ is plotted as a function of $\Delta A / [L]_f$ and the slope is $-1/K_{app}$.

The values for the equilibrium constants for axial ligation with respect to unprotonated ligand Eq.(8) were calculated from the relation $K_{eq} = K_{app} / \alpha_L$

$$K_{eq} = \frac{[RCo(DH)_2L][RCo(DH)_2H_2O][L]}{[RCo(DH)_2H_2O+L]} \ldots (9)$$

where $\alpha_L$ (fraction of ligand as free base) was calculated from Eq.(10)

$$\alpha_L = K_a / \left( K_a + [H^+] \right) \ldots \ldots (10)$$

$K_a$ is the dissociation constant of the ligand.

**Kinetic measurements**

Kinetic measurements were made for the binding of imidazoles to bromomethyl(aquo)cobaloxime. For
Each ligand L, at various 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, in at least 10 fold excess over cobaloxime concentration (0.00125 mol dm\(^{-3}\)).

Reaction progress was monitored by measurements of the change in the absorbance upon addition of bromomethyl(aquo)cobaloxime to a 3 ml cuvette which contained KCl to maintain unit ionic strength, and necessary buffer (0.2 mol dm\(^{-3}\)) to maintain pH and ligand in the thermostated (25 ± 0.1°C) cell compartment of Elico SL 171 model.

First order rate constants \(k_{\text{obs}}\) were obtained by least-squares fits of the data to Eq. (11)

\[
\ln (A_t - A_\infty) = k_{\text{obs}} t \tag{11}
\]

where \(A_t\) is the absorbance at time \(t\) and \(A_\infty\) is the final absorbance.

Second order rate constants, \(k_{\text{on}}\) at a given pH for a given ligand were obtained from the slopes of least squares fits of the data to Eq. (12)

\[
k_{\text{obs}} = k_{\text{on}} [L] + k_{\text{off}} \tag{12}
\]

where \([L]\) is the total concentration of L present. Values of \(k_{\text{on}}\), the second order ligation rate constant with respect to free ligand base were calculated from \(k_{\text{on}} = k_{\text{on}'} / c_{\text{L}}\).

Determination of second order rate constant is exemplified by axial ligation of 1-Me IMD to bromomethyl(aquo)cobaloxime at pH = 4.5 and 25 ± 0.1°C.

The plot of \(k_{\text{obs}}\) versus concentration of ligand is linear and the slope of the plot gives second order rate constant \(k_{\text{on}}\).

Results and Discussion

Figure 1 shows the pH dependence of binding of imidazole to BrCH\(_2\)Co(DH\(_2\))OH\(_2\). For the same concentration of imidazole, as the pH is increased the binding constant increases (In Fig. 2 the absorbance decreases with increase in pH). Figure 3 shows the association kinetics as the time increases the
absorbance decreases indicating the formation of complex whereas Fig. 4 shows the increase in absorbance with increase in time, this is because the complex [BrCH₂Co(DH)₂]OH₂ dissociates and forms BrCH₂Co(DD)₃OH₂ with increase in time.

The equilibrium constants for the ligation of BrCH₂Co(DD)₃OH₂ by imidazoles is dependent upon the $pK_a$ values of the conjugate acid of the ligands. As the pH increases the apparent binding constants increase (Table 1). The $pH$ dependence of the apparent binding constants for ligation, $K_{app}$ from pH 4.5 to 8.0 is consistent indicating that IMD free base is the sole ligating species. As the pH is decreased, $K_{app}$ decreased. The dependence of $pH$ on the binding of imidazoles to BrCH₂Co(DD)₃OH₂ is shown in Fig. 1. This may be because of the competition of $H^+$ with Co(III) to bind with imidazole. Hence, at lower pH most of the imidazole is protonated and not available for binding with cobalt. At higher pH imidazole (free base) available is maximum and binds to Co(III). So the $K_{app}$ is larger at higher pH. At pH 8.0 and above imidazole is completely in the form of free base and completely available for binding hence, the binding is $pH$ independent. Similar trends were observed with 1-Me imidazole. In these two cases the $pH$ dependent binding constants were measured from 4.0 to 10.0 $pH$ to demonstrate the $pH$-dependent and $pH$-independent binding of these ligands to BrCH₂Co(DD)₃OH₂. Whereas in the case of 2-Me IMD, 2-Et IMD and 1,2-DM IMD, the binding constants could not be measured below $pH$ 7.0 as they

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Table 1—Log $K_{app}$ of various imidazoles for the axial ligation of alkyl(aquo)cobaloximes various $pH$ at 25°C

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<th>2-Et IMD</th>
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Table 2—$pH$-dependence of $k_{obs}$ of various imidazoles for the axial ligation of alkyl(aquo)cobaloximes at 25°C

bind weakly to Co(III) of cobaloxime. For these systems the binding is $pH$-dependent up to $pH$ 8.5 and above $pH$ 8.5 the binding is independent of $pH$. The order of stability with respect to imidazole is 1-Me IMD > IMD > 2-Me IMD > 2-Et IMD. In the case of 1-Me IMD and IMD the stabilities increased with increase basicity. Whereas from IMD to 2-Et IMD though the basicity increases the binding constants decreases. This may be attributed to the increase in steric hindrance due to the presence of methyl or ethyl group at C₂ of imidazole.

Similar trends were observed in the substitution reactions of CH₂Co(DD)₃OH₂ with P(n-But)₃ or pyridine. Though P(n-But)₃ is more basic and greater π back-bonding ability than pyridine, tributyl phosphine reacts more slowly and forms less stable complex than pyridine. It is expected that P(n-But)₃ would react faster due to its high basicity and back-bonding ability. This can be explained based on the steric hindrance, since P(n-But)₃ is more bulky than pyridine it forms less stable complex. If we compare the binding constant$^{16,17}$ of various ligands with BrCH₂Co(DD)₃OH₂ they are in the order $K_{OH} < K_{py} < K_{IMD} < K_{1-MeIMD} < K_{CN}$. This trend is in accordance with the basicity and also with increase in π back-bonding (Co(III)→L) in the above series. In addition, the much higher stability of CN⁻ compared to pyridine and imidazole is explained on the basis of following reasons: i) Cyanide is more basic than IMD and pyridine; ii) cyanide is a better π acceptor than imidazole and pyridine; and iii) cyanide is a soft base whereas imidazole and pyridines are borderline bases.

Co(III) in cobaloximes is a soft acid, the soft-soft interaction between the Co(III) of cobaloxime and
CN− facilitates the stronger and more stable complex formation. Though P in P(n-But)3 is soft15 and more basic forms less stable complex than IMD and pyridine indicating that steric hindrance plays a dominant role. Similar trends were observed18 in the study of [CN Co(DH2)L] where L = 1-Me IMD, IMD, 2-Me IMD or 2-Et IMD. The C=N stretching frequencies increased because of steric hindrance18.

The plot of pseudo-first order rate constant $k_{obs}$, against ligand concentration was linear with very small intercept indicating that some dissociation accompanies the complex formation. It appears to be more likely at low pH (i.e. below the $pK_a$ of the ligand) probably due to the protonation of ligand. This could be supported on the basis of low binding constant at the pH values below the $pK_a$ of ligand (Table 1). The kinetic studies could not be taken up at high pH (beyond pH 7.0) by the conventional methods. This aspect could also be supported with observed high binding constant values measured under similar conditions. For 1-Me IMD and IMD after pH 6 there is a sharp raise in the $k_{obs}$, whereas for other imidazoles, there is only a small increase in $k_{obs}$ with increase in pH between 7.5 and 9.0 (Table 2). These kinetic data are supported by binding studies. This is demonstrated in Fig.5 that rate of formation ($k_{obs}$) is influenced by the steric factor and not by the basicity of the ligands (Table 3). As the pH is increased the rate of formation of complex increases, at the same time at any given pH as the concentration of imidazole increases (for a fixed concentration of BrCH2Co(DH2OH2) the $k_{obs}$ increases (Fig.6). The rate of formation of complex for various imidazoles increases in the order 1-Me IMD > IMD > 1,2-DM IMD > 2-Me IMD > 2-Et IMD. When we compare the dissociation constants the ease of dissociation of imidazole from the complex is in the order 2-Et IMD > 2-Me IMD > 1,2-DM IMD > IMD > 1-Me IMD.

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References


Table 3—$k_{obs}$ of various imidazoles for the axial ligation of alkyl(aquo)cobaloximes at 25°C

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<th>$k_{obs}$</th>
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Fig. 5—Dependence of log $k_{obs}$ on $pK_a$ of various imidazoles at 25°C.

Fig. 6—Dependence of $k_{obs}$ on the pH of various imidazoles at 25°C for the axial ligation of bromomethyl(aquo)cobaloxime.