Coenzyme B₁₂ model studies: Equilibria and kinetics of axial ligation of trifluoroethyl(aquo)cobaloximes by N donor ligands

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Equilibria and kinetics of the reaction of trifluoroethyl(aquo)cobaloxime with imidazole and substituted imidazoles have been studied as a function of pH at 25°C, 1.0 M ionic strength (KCl) by UV-vis spectrophotometry. Comparison of equilibrium constants with respect to ligand are in the order $K_1$-Me> $K_1$-ind> $K_2$-Me> $K_1$-2-Diim> $K_2$-ind which are correlated to basicity and steric factors. The equilibrium constants are correlated to the softness and ability to form dπ-pπ back bonding.

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Imidazole is a typical representative of a large group of heterocyclic nitrogen donor atom ligands. Imidazole and its derivatives are components of a variety of biologically important molecules, where they play the role of ligands and mainly for this reason coordination chemistry of these species is still under development. Imidazole normally coordinates the metal ions through its pyridine nitrogen, although it is potentially an ambidentate ligand, which can also bind via the pyrrole nitrogen.

The understanding of the chemistry of cobalamins requires extensive studies of several types of model systems since the differences in properties between the models and cobalamins are instructive. If changes in the nature of the ligands in a particular type of model compound lead to properties more similar to those of cobalamins, then such information is of particular value.

In order to understand better the structure and reactivity of cobalamins, simple models have been proposed and investigated. Model complexes for vitamin B₁₂ have played an important role in understanding the behaviour of ligand substitution of vitamin B₁₂ and the role of cobalt-carbon bond in coenzyme B₁₂. Equilibria and kinetics of axial ligation of alkyl(aquo)cobaloximes with imidazoles and substituted imidazoles were studied. Recently ligand substitution reactions of iodocobalamin was reported by Marques et al. Kofod synthesized simple model for coenzyme B₁₂, which undergoes a rapid ligand substitution reaction with ethylenediamine to form trans-[Co(en)₂MeNH₃]²⁺.

Equilibria and kinetics for axial ligation of bromomethyl(aquo)cobaloxime and binding studies of various pyridines with methyl and ethyl(aquo)cobaloximes were reported by Bhoopal et al.

To provide further information concerning the ligand substitution reactions we have studied binding constants for a series of imidazole containing ligands. These were chosen because (i) they form stable complexes with cobaloximes; (ii) ligands with a wide range of $pK_a$ values; (iii) significant spectroscopic changes occur in their coordination so that reactions are readily followed spectroscopically; and (iv) their coordination chemistry is expected to be simple.

Materials and Methods

Imidazoles and substituted imidazoles, were obtained from Sigma and were used without further purification. KCl, HPLC grade methanol, acetic acid, HCl, phosphoric acid, formic acid were obtained from Fluka. Dipotassiumhydrogen phosphate, potassium dihydrogen phosphate, potassium phosphate, tris, sodium acetate and potassium hydroxide were obtained from Acros. Double distilled, deionized water was used throughout.

To maintain appropriate pH 0.2M buffers of HCl (pH=0-1.5), KH₂PO₄ and H₃PO₄ (pH = 2.0), HCOOH and KOH (pH = 2.5-3.0), CH₃COOH and CH₃COONa (pH = 3.5-5.5), K₃HPO₄ and KH₂PO₄ (pH = 6.0-8.0), Tris and HCl (pH = 8.5-9.0), K₂HPO₄ and K₃PO₄ (pH = 9.5-11.5) are used.

Alkyl(aquo)cobaloxime was prepared by the procedure of Brown et al. All manipulations were performed under minimal illuminations. These alkyl(aquo)cobaloximes are photo labile.
particularly in solution. These are soluble in alcohols 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 aluminium foil. All 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 pH = 9.2) with standard buffer solutions.

UV and visible spectra were recorded on a Hitachi U-3410, the sample compartment of which is provided with a thermostat and the concentrations of trifluoroethyl(aquo)cobaloxime (0.00133 M) was fixed at 438 nm.

For axial ligation 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

Values for the $K_a$ of the conjugate acid of ligands (scheme 1) were obtained by potentiometric titration at 25 ± 0.1°C. Values of $pK_a$'s were obtained by algebraic method from Eq. (1)

$$K_a = [L] [H^+] /[HL] \quad \ldots (1)$$

In a 3 ml cuvette solutions containing RCo(DH)$_2$(OH$_2$) appropriate buffer (0.2 M) to maintain pH, KCl to maintain ionic strength (1.0 M) and varying concentrations of ligand were taken in a cell maintained at 25 ± 0.1°C. Solutions were allowed to temperature – equilibrate in the spectrophotometer cell block for at least 15 min, prior to addition of cobaloxime.

$$K_{app} = \frac{[RCo(DH)_2L]}{[RCo(DH)_2H_2O][L]} \quad \ldots (2)$$

Scheme 1

Final absorbance readings were taken after equilibrium was established as indicated by the time independence of the readings. At a given pH, Eq. (3) is applied

$$\Delta A = \frac{\Delta A_{\text{max}}[L]_f}{(1 + K_{app} [L]_f)} \quad \ldots (3)$$

where $\Delta A$ is the difference in absorbance between solutions containing cobaloxime and added ligand and solutions containing only cobaloxime at the same concentration, $\Delta A_{\text{max}}$ is the maximum absorbance change thus obtained at high [L], and [L]$_f$ is free ligand concentration. The data were analysed by a least-squares fit to the rearranged form of Eq. (3) to give Eq. (4)

$$\Delta A = \Delta A_{\text{max}} \left[ 1/K_{app} \Delta A/[L]_f \right] \quad \ldots (4)$$

$$[L]_f = [L]_{\text{tot}}(C_T \Delta A/\Delta A_{\text{max}}) \quad \ldots (5)$$

$[L]_f$ is calculated from Eq. (5) using measured value of $\Delta A_{\text{max}}$, where [L]$_{\text{tot}}$ is the total concentration of added ligand and $C_T$ is the total concentration of cobaloxime. Values of $K_{app}$ were obtained from the least-squares fit of Eq. (4) i.e., the plot of $\Delta A$ versus $\Delta A/[L]_f$ where the slope is $K_{app}$. The pH independent equilibrium constant ($K_{eq}$) is calculated using the relation $K_{eq} = K_{app}/C_T$, where $C_T$ is the fraction of the ligand present as free base (unprotonated) and is calculated from Eq. (6).

$$C_T = \frac{K_a}{(K_a + [H^+] )} \quad \ldots (6)$$

Kinetic measurements

For each ligand L, at various pH, first order rate constants ($k_{obs}$) were determined from the absorbance measurements at the same wavelength used for $K_{app}$ determinations under pseudo-first order condition with L, in at least 10 fold excess over cobaloxime concentration (0.00133 mol dm$^{-3}$). Reaction progress was monitored by measuring the change in absorbance upon addition of trifluoroethyl(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. First order rate constants ($k_{obs}$) were obtained by least-squares fits of the data to Eq. (7)

$$\ln A_t - \ln A_{\text{eq}} = k_{obs} t \quad \ldots (7)$$
where $A_t$ is the absorbance at time $t$ and $A_0$ is the final absorbance.

Second order rate constants, $k_{\text{obs}}$, at a given $p$H for a given ligand were obtained from the slopes of least squares fits of the data to Eq. (8) by plotting $k_{\text{obs}}$ vs [ligand].

$$k_{\text{obs}} = k_{\text{on}} [L]_T + k_{\text{off}}$$ \hspace{1cm} (8)

where $[L]_T$ is the total concentration of $L$ present. The plot of $k_{\text{obs}}$ versus $[\text{ligand}]$ is linear and the slope of the plot gives second order rate constant ($k_{\text{on}}$). Values of $k_{\text{on}}$ the $p$H independent second order ligation rate constant with respect to free ligand base were calculated from Eq. (9)

$$k_{\text{on}} = k_{\text{on}} / \alpha_L$$ \hspace{1cm} (9)

**Results and Discussion**

$^1$H NMR spectra of CF$_3$CH$_2$Co(DH)$_2$OH$_2$ in DMSO-$_d_6$ show two peaks one at 2.09 ppm corresponding to four equivalent methyl groups, the other at 3.30 ppm belonging to CF$_3$CH$_2$ protons. The complex formation constants for the substitution of water on CF$_3$CH$_2$Co(DH)$_2$OH$_2$ by CN$^-$, imidazole 1-methyl imidazole, 2-methyl imidazole, 1,2-dimethyl imidazole and 2-ethyl imidazole were determined in the following manner. The complex concentration was kept constant at $1.00 \times 10^{-3}$ M and different concentrations of the ligand, $L$, were added

$$\text{CF}_3\text{CH}_2\text{Co(DH)}_2\text{OH}_2 + L \rightarrow \text{CF}_3\text{CH}_2\text{Co(DH)}_2\text{OH}_2L + \text{H}_2\text{O}$$ \hspace{1cm} (10)

On increasing [ligand] the characteristic peak at 438 nm is incrementally reduced and almost disappears completely at high ligand concentrations.

The 1:1 complex formation constants for CN$^-$, 1-MeIMD, IMD, 2-MeIMD, 1,2 DiMeIMD and 2-EtIMD were determined and reported in Table 1. A plot of complex formation constants ($\log K_{\text{app}}$) vs $p$H is shown in Fig. 1.

It can be seen that $\log K_{\text{app}}$ increases linearly with increasing $p$H of the solution. When $K_{\text{eq}}$ values of various ligands are compared, they are in the order CN$^-$>$1$-MeIMD$>$IMD$>$2-MeIMD$>$1,2-DiMeIMD$>2$-EtIMD. From CN$^-$ to IMD they are in accordance with $pK_a$ of the ligand; as the $pK_a$ increases the $K_{\text{eq}}$ increases, whereas from IMD to 2-EtIMD though $pK_a$ of the ligand increases the $K_{\text{eq}}$ decreases. This is because of the steric hindrance caused by the substituent at the 2 position of imidazole.

The equilibrium constants for the ligation of CF$_3$CH$_2$Co(DH)$_2$OH$_2$ by imidazoles is dependent upon the $pK_a$ values of the ligands. The $p$H dependence of the apparent binding constants for ligation, $K_{\text{app}}$ from $p$H 4.5 to 8.0 is consistent indicating that IMD free base is the sole ligating species. As the $p$H is decreased $K_{\text{app}}$ decreased. The dependence of $p$H on the binding of imidazoles to CF$_3$CH$_2$Co(DH)$_2$OH$_2$ is shown in Fig. 1. This may be because of the competition of $H^+$ with Co(III) to bind with imidazoles. Hence, at lower $p$H most of the imidazole is protonated and not available for binding with cobalt. At higher $p$H imidazole available is maximum and binds to Co(III). So the $K_{\text{app}}$ is larger at higher $p$H. At $p$H 8.0 and above imidazole is completely in the form of free base and completely available for binding and hence the binding is independent of $p$H. Similar trends were observed with 1-Me imidazole. In these two cases the $p$H dependent binding constants were measured from $p$H 5.0 to 8.5

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$K_{\text{eq}}$ values are tabulated in Table 1.
to demonstrate the pH dependent and pH independent binding of these ligands to CF₃CH₂Co(DH)₂OH₂, whereas in the case of 2-Me IMD, 2-Et IMD and 1,2 DiMe IMD the binding constants could not be measured below pH 6.5 as they bind weakly to Co(III) of cobaloxime. For these systems the binding is independent of pH. If we compare the binding constants of various ligands with CF₃CH₂Co(DH)₂OH₂ they are in the order $K_{\text{OH}} < K_{\text{Py}} < K_{\text{IMD}} < K_{\text{Me IMD}} < K_{\text{CN}}$. This trend is in accordance with the basicity and also with increase in $\pi$ back-bonding (Co(III) $\rightarrow$ L) in the above series. In addition, the much higher stability of CN$^-$ compared to pyridine and imidazole could be explained on the basis of i) cyanide is more basic than IMD and pyridine, ii) cyanide is a better $\pi$ acceptor than imidazole and pyridine, and iii) cyanide is a soft base whereas imidazole and pyridine are border line bases. Co(III) in cobaloximes is soft acid, the soft-soft interaction between the Co(III) of cobaloxime and CN$^-$ facilitates the stronger and more stable complex formation. Though 2-MeIMD, 1,2DiMeIMD and 2-EtIMD are more basic than pyridine and IMD, they form less stable complexes, this is due to steric hindrance, similar trends were observed in the study of [CNCo(DH)₂L] (where L=2-substituted imidazoles) and in the binding of P(n-but)$_3$ to cobaloximes. Though the P in P(n-but)$_3$ is soft and more basic than imidazoles, the binding is weak, indicating that steric hindrance plays a dominant role.

Walker has reported that log$K$ vs. $pK_a$ plots are linear for the coordination of imidazole, pyridine and non-aromatic cyclic amines to a cobalt porphyrine. The coordination of an imidazole was found to be stronger than a pyridine, which in turn is stronger than a non-aromatic amine. The author interpreted this with $d\pi$-$\pi$ back bonding, the added stability of the imidazole complexes over the pyridine compounds being due to higher basicity and greater $d\pi$ back bonding in the imidazole system. The same argument was applied to the comparison of the pyridine and non-aromatic amine complexes.

The concept of metal to ligand $\pi$ bonding will explain both the order of strength of ligation and the reverse order for the dependence of ligation strength upon ligand basicity. The order of CF₃CH₂Co(DH)₂L stability is attributed to the ability of imidazoles to accept electrons into higher energy unfilled $\pi^*$ antibonding orbitals. This ligand stability order is the same as the order previously established for the trans direction of substituents into the square-planar complexes of Pt$^{3+}$ and for the stabilization of low valent states of numerous transition metals, all these have been related to the $\pi$-accepting ability of the ligands.

We have also carried out association, dissociation and concentration dependent kinetics with CN$^-$, IMD and 1-MeIMD (Tables 2 and 3). Association rate constants given in Table 2 clearly indicates that as the pH increases the rate of complex formation also increases. The increase in the rate constants can be attributed to increase in the available amount of corresponding ligand in deprotoned form which is generally high at higher pH. The above argument also
In concentration dependent kinetics it is observed that as the concentration of the ligand increases, the rate constants also increased. The pH dependent second order rate constants were determined by plotting the pseudo-first order rate constants vs concentration of the ligand. The intercept of the linear plots was not significantly different from zero (Fig. 2), slope of this linear plot is the pH dependent second order rate constant \( k_{off} \). For better comparison the pH independent second order rate constants \( k_{on} \) have been calculated and observed that the order obtained for rate constants and in binding constants are the same.

**Conclusions**

The order of binding of imidazole and substituted imidazoles to \( \text{CF}_3\text{CH}_2\text{Co(DH)}_2\text{OH}_2 \) is explained based
on basicity and steric hindrance. Though the basicity of 2-Melmd, 1,2-Dimeimd and 2-Ethylimd is more basic than IMD, they form less stable complexes due to steric hindrance.

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References