Proc. Indian Acad. Sci. (*Chem. Sci.*), Vol. 115, No. 2, April 2003, pp 83–90

Equilibria and kinetics for pH-dependent axial ligation of bromomethyl(aquo)cobaloxime by aliphatic amine ligands

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MS received 27 July 2002; revised 16 October 2002

Abstract. Kinetics and equilibria of axial ligation of bromomethyl(aquo) cobaloxime by a series of straight chain primary amines (methylamine, ethylamine, propylamine, butylamine, pentylamine, hexylamine), cycloamines (cyclopentylamine, cyclohexylamine, cycloheptylamine) and secondary amines (N,N-dimethylamine, N,N-diethylamine) have been measured as functions of pH by spectrophotometric technique in aqueous solution, ionic strength 1 M (KCl) at 25°C. The rate of substitution of H_2O varies with the p*Ka* of incoming ligand, thus establishing nucleophilic participation of the ligand in the transition state. Binding and kinetic data are interpreted based on the basicity and steric influence of the entering ligand. To compare the rate constants of the entering ligands, pH independent second-order rate constants (k_{on}) are calculated.

Keywords. Alkylcobaloximes, axial ligation; bromomethyl(aquo)cobaloxime; cycloamines.

1. Introduction

B_{12}-based enzymes are among the few cofactors known so far that contain a metal-carbon bond. These coenzymes belong to the alkylcobalamin series[1,2] (RCbl). All the B_{12} enzymatic reactions involve the making and breaking of the Co–C bond. In order to understand better the structure and reactivity of cobalamins, simple models have been proposed and investigated.[3–7] Such model studies provide the clues to the elusive mechanisms of B_{12}-dependent enzymatic processes particularly those involving the homolytic Co–C cleavage. Model complexes for vitamin B_{12} have played an important role in understanding the behaviour of ligand substitution of vitamin B_{12} and the role of the Co–C bond in the coenzyme B_{12}.[8,9] Many octahedral organometallic complexes of Co(III) such as cobalamins and Costa complexes[10,11] have been suggested as models for the vitamin B_{12} coenzyme to mimic either the nature of the active site or a specific function of the enzyme. The study of simple models such as cobaloximes, $RCo(DH)_2L$, where L = neutral ligands, R = alkyl groups and DH = dimethylglyoximato mono anion, has furnished a significant amount of data.[12,13] This has provided some foundation for understanding the behaviour of cobalamins.[14]

A few cobaloximes have been subjected to extensive kinetics and mechanistic studies.[15–17] Recently, the equilibria and kinetics of axial ligation of alkyl(aquo)cobal-

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oximes with imidazole and substituted imidazoles were studied.[18–21] Compared to both cobalamins and other model systems, cobaloximes have stronger Co–C bonds[22] and shorter Co-L (L = pyridine, substituted pyridines) bonds.[13] Many model complexes[23–28] have shown that the ligand in the *trans* position to the Co–C bond can affect the kinetics and thermodynamic stability of the bond. In view of this, we have studied the reaction of various linear chain primary amines, secondary amines and cyclo amines with bromomethyl(aquo)cobaloxime.

2. Materials and methods

Methylamine, ethylamine, propylamine, butylamine, cyclohexylamine and cyclo heptylamine were obtained from S.D. Fine chemicals. Pentylamine and hexylamine were obtained from Merck (Germany). KCl and HPLC grade methanol were obtained from Fluka. Dipotassium hydrogenphosphate, potassium dihydrogenphosphate, potassium phosphate, tris(hydroxymethyl)- aminomethane (Tris), sodium acetate, potassium hydroxide were obtained from Across. Double-distilled and deionised water was used throughout.

Bromomethyl(aquo)cobaloxime was prepared by the procedure of Brown *et al*.[29] All manipulations were performed under minimal illumination due to photolability of the carbon–cobalt bond.[30]

pH values were determined with a Digisun digital pH-meter equipped with a combined glass electrode. The electrode was standardized at two pH values (4 and 9·2) with standard buffer solutions. UV-visible spectra were recorded on a Hitachi U-3410 spectrophotometer, the sample compartment of which is provided with a thermostat. Bromomethyl(aquo)cobaloxime (0·001 M) absorption was fixed at 436 nm. For monitoring axial ligation kinetics, single wavelength measurements were made on an Elico single beam spectrophotometer SL 171 model, the sample compartment of which was thermostatted at 25 ± 0·1°C.

3. Results and discussion

3.1 *Determination of dissociation constant of the ligands*

Values for the p*Ka* of the conjugated acids of ligands were obtained by potentiometric titrations at 25 ± 0·01°C, using a linear least-squares fit of the data to (1) below, where a_L is the fraction of the total ligand present as the free base (see (2) and (3)).

$$\mathrm{pH} = \mathrm{p}Ka + \log\,[(a_L)/(1-a_L)], \tag{1}$$

$$\mathrm{p}Ka = [\mathrm{L}][\mathrm{H}^+]/[\mathrm{HL}], \tag{2}$$

$$a_L = Ka/(Ka + [\mathrm{H}^+]). \tag{3}$$

Ka is the dissociation constant of the ligand.

3.2 *Determination of equilibrium constant*

Apparent equilibrium constants (K_{app} values, (4) below) for the axial ligation of bromomethyl(aquo)cobaloxime was determined by spectrophotometric measurements. Solutions containing $BrCH_2Co(DH)_2(OH)_2$, an appropriate buffer (0·2 M) to maintain pH, KCl to maintain ionic strength (1·0 M) and varying concentrations of ligand, are taken in

3 ml cuvettes and allowed to equilibrate in a thermostatted cell compartment holder at 25 ± 0·1°C for 15 min prior to addition of $BrCH_2Co(DH)_2OH_2$.

$$K_{app} = \frac{[BrCH_2Co(DH)_2L]}{[BrCH_2Co(DH)_2H_2O][L]_{free}}. \quad (4)$$

Final absorbance readings are taken after equilibrium is established as indicated by the time independence of the readings. For such experimental setup, at a given pH, K_{app} is calculated from the experimental data as,

$$\Delta A = \Delta A_{max}[L]_f/[(1/K_{app} + [L]_f], \quad (5)$$

where ΔA is the difference in absorbance between solutions containing cobaloxime + added ligand (L) and solutions containing only cobaloxime at the same concentration, ΔA_{max} is the maximum absorbance change thus obtained at high $[L]_T$, and $[L]_f$ is the unbound ligand concentration. The data are analysed by a least-squares fit to the rearranged form of (5) to give (7)

$$[L]_f = [L]_T - (C_T\Delta A/\Delta A_{max}), \quad (6)$$

$$\Delta A = \Delta A_{max}\{(1/K_{app})(\Delta A/[L]_f)\}. \quad (7)$$

$[L]_f$ is calculated from (6) using the measured value of ΔA and ΔA_{max}, $[L]_T$ is the total concentration of added ligand and C_T is the total concentration of cobaloxime. Values of K_{app} are obtained from the least-squares fit of (7) i.e. the plot of ΔA vs $\Delta A/[L]_f$ and the slope is $-1/K_{app}$. The pH independent binding constant K_{eq} is calculated from the relation $K_{eq} = K_{app}/a_L$, where α_L is calculated from (3).

All the ligands which are chosen for this study undergo protonation of nitrogen atom with acid dissociation constants p*Ka* values in the range of 10·4 to 11. Figure 1 shows the concentration dependence of binding of ethylamine to $BrCH_2Co(DH)_2OH_2$. As the concentration of ethylamine increases the absorbance decreases. The values of equilibrium constant and K_{app} for the reaction of all the above ligands with bromomethyl(aquo)cobaloxime are given in table 1. Logarithmic plots of log K_{app} vs pH are shown in figure 2, which indicates that as the pH increases the K_{app} values increase and the affinity for ligands increases in the order: N,N-diethylamine < N,N-dimethylamine < methylamine < ethylamine < propylamine < butylamine < pentylamine < hexylamine in straight chain amines and cycloheptylamine > cyclohexylamine > cyclopentylamine in cycloamines.

If we observe pH-dependent binding plots (figure 2), K_{app} values increase up to a certain values of pH and after this they are pH independent. Similar trends are observed for all the ligands studied. Due to the negative inductive effect, electron density on nitrogen of amines increases as the carbon chain increases, i.e. electron-donating ability increases and hence the availability of the pair of electrons on nitrogen for bonding increases. Though the secondary amines N,N-dimethylamine and N,N-diethylamine are more basic than the primary amines, ethylamine and methylamine, the secondary amines form less stable complexes than the corresponding primary amines. This is due to steric hindrance. Amongst the secondary amines, though the diethylamine is more basic than the dimethylamine, it forms less stable complexes as due to the bulky ethyl groups on nitrogen. This clearly indicates that the steric crowding on nitrogen decreases the ability of the amine nitrogen to bind Co(III). The equilibrium constant values of normal chain amines are greater than the corresponding cyclo amines because straight chain amines are more basic than corresponding cyclo amines.

3.3 *Determination of rate constants (k_{on})*

For each ligand L, at various pH values, first-order rate constants (k_{obs}) are determined from the absorbance measurements at the same wavelength used for K_{app} determination. Under pseudo-first order condition, concentration of L was at least in 10-fold excess over $BrCH_2Co(DH)_2OH_2$ concentration. Reaction progress is monitored by measurements of the change in the absorbance upon addition of $BrCH_2Co(DH)_2OH_2$ to a 3 ml cuvette,

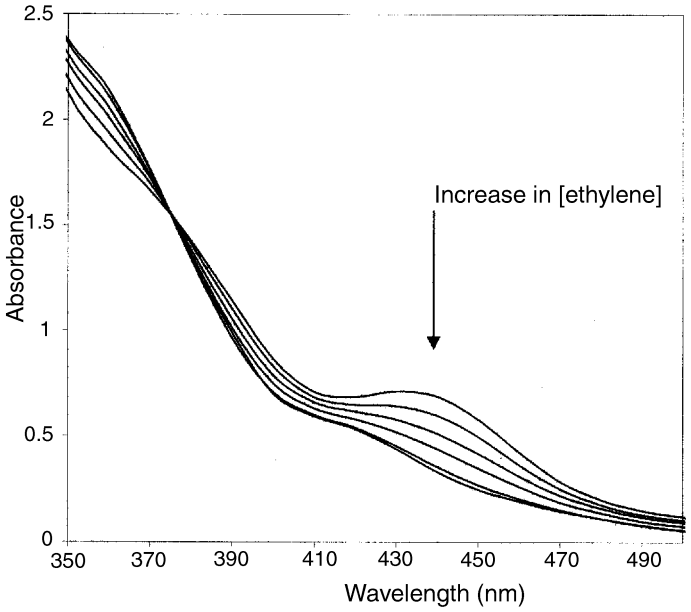

Figure 1. UV-visible scan for $BrCH_2Co(DH)_2OH_2$ with varying concentrations of ethylamine at pH 10·5 and 25°C.

Table 1. Formation constants (log K_{app} and K_{eq} for the axial ligation of $BrCH_2Co(DH)_2OH_2$ by L at 25°C.

	log K_{app} at pH =								
L	8·0	8·5	9·0	9·5	10·0	10·5	11·0	11·5	K_{eq}
CH_3–NH_2	0·652	1·150	1·641	2·116	2·544	2·872	3·064	3·148	1563·60
C_2H_5–NH_2	0·708	1·205	1·700	2·176	2·615	2·959	3·170		2058·0
C_3H_7–NH_2	0·843	1·341	1·835	2·315	2·760	3.119	3·347	3·454	3267·30
C_4H_9–NH_2	0·930	1·430	1·924	2·407	2·860	3·237	3·487	3·610	4804·6
C_5H_{11}–NH_2	0·937	1·437	1·932	2·418	2·876	3·266	3·533	3·688	5616·70
C_6H_{13}–NH_2	0·958	1·457	1·953	2·441	2·904	3·305	3·588	3·737	6750·4
CHPA		0·728	1·222	1·706	2·159	2·536	2·786	2·909	956·1
CHA		0·706	1·201	1·680	2·123	2·480	2·705	2·810	739·98
CPA	0·217	0·714	1·203	1·670	2·079	2·376	2·538	2·604	436·12
N,N-DMA			0·900	1·381	1·826	2·188	2·419	2·527	388·12
N,N-DEA				0·760	1·226	1·633	1·937	2·101	160·23

CHPA = cycloheptylamine, CHA = cyclohexylamine, CPA = cyclopentylamine, N,N-DMA = N,N-dimethylamine, N,N-DEA = N,N-diethylamine. Values are accurate up to ± 0.05

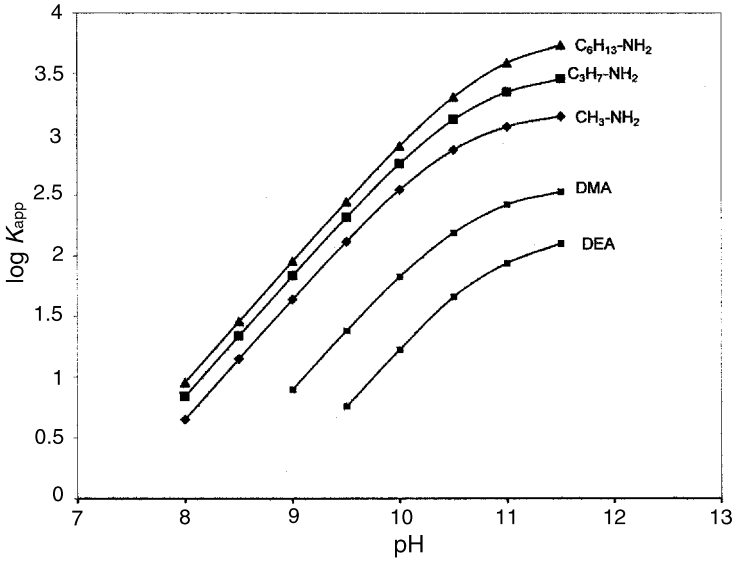

Figure 2. Dependence of log K_{app} on pH for the formation of $BrCH_2Co(DH)_2L$ at 25°C. DMA = N,N-dimethylamine, DEA = N,N-diethylamine.

containing KCl to maintain ionic strength, necessary buffer (0·2 M) to maintain pH and ligand in the thermostatted (25 ± 0·1°C) cell compartment. First-order rate constants (k_{obs}) are obtained by least-squares fits of the data as,

$$\ln(A_t - A_\infty) = k_{obs}t, \tag{8}$$

where A_t is the absorbance at time t and A_∞ is the final absorbance.

Second-order rate constants, k'_{on}, at a given pH for a given ligand are obtained from the slopes of least squares fit of the data as below,

$$k_{obs} = k'_{on}\,[L]_T + k_{off}\,, \tag{9}$$

where $[L]_T$ is the total concentration of ligand present (table 2). Values of k_{on} the pH independent second-order rate constant, are calculated from $k_{on} = k'_{on}/a_L$, where a_L is defined above.

The plots of pseudo first-order rate constant k_{obs} against concentration of corresponding amines is linear with a very small intercept (figure 3), which may indicate that a small dissociation is accompanied by the complex formation. This dissociation is more likely at lower pH and is probably due to the protonation of the ligand. Plots of k_{obs} vs pH are sigmoidal, i.e. from pH 9·5 to 10·5, k_{obs} increases slowly and then increases suddenly from 10·5 to 11 pH after which it is steady and there is no change in k_{obs}. Values of k_{obs} at various pH values for different ligands are given in table 3 and representative plots are shown in figure 4. Figure 5 shows the time-dependent binding of ethylamine to $BrCH_2Co(DH)_2OH_2$ at pH 10·5.

Kinetics of substitution of the axial base in alkylcobaloximes and related cobalt complexes has been studied under a variety of conditions.[31,32] In none of these studies however the mechanism has been established conclusively although in all cases strong evidence has been provided that the intimate mechanism is dissociative (id). In coordinating solvents, 'penta-coordinate' species are found involving penta-coordinate

alkyl cobalt complexes and solvent. In view of the evidence presented above for the existence of penta-coordinate alkylcobaloximes and the ligation kinetic studies by others,[33–35] an *SN*[1] mechanism appears to be operative in the present case. This is also supported by Marques *et al*[36] in the ligand substitution reactions of iodocobalamin. The small dependence of k_{on} upon ligand basicity within each series of ligands is clearly

Table 2. Kinetic data for the ligation of bromomethyl(aquo)cobaloxime at 25°C.

Amine	k'_{on} ($dm^3\ mol^{-1}\ s^{-1}$)	*a*	k_{on} ($dm^3\ mol^{-1}\ s^{-1}$)
Methylamine	0·102	0·0836	1·22
Ethylamine	0·108	0·0720	1·50
Propylamine	0·130	0·0633	2·05
Butylamine	0·134	0·0532	2·5
Pentylamine	0·150	0·0490	3·23
Hexylamine	0·208	0·0409	5·09
Cycloheptylamine	0·064	0·0532	1·20
Cyclohexylamine	0·064	0·0647	0·99
Cyclopentylamine	0·0516	0·0872	0·59

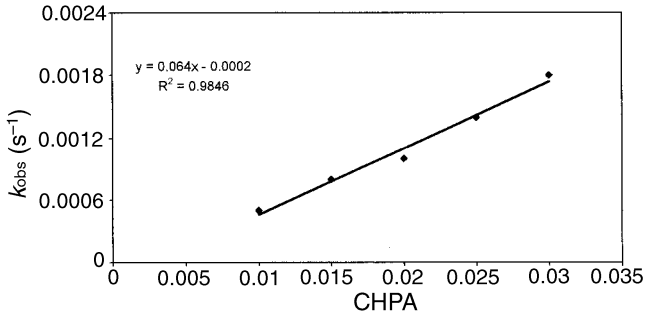

Figure 3. Dependence of [cycloheptylamine] on the pseudo first-order rate constant (k_{obs}) for the formation of $BrCH_2Co(DH)_2CHPA$ at 25°C, the gradient k_{on} = 0·064 $dm^3\ mol^{-1}\ s^{-1}$.

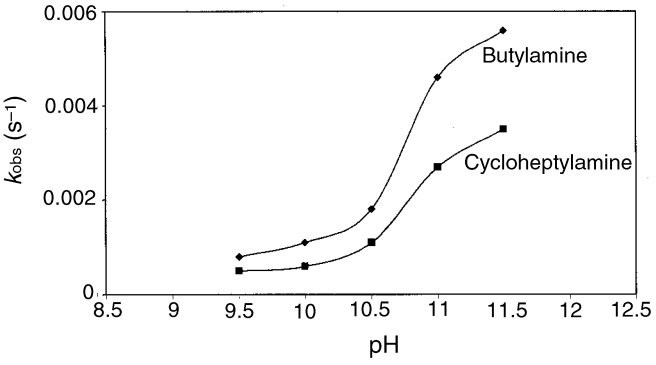

Figure 4. Dependence of k_{obs} on pH for the axial ligation of $BrCH_2Co(DH)_2OH_2$ by butylamine and cycloheptylamine.

Table 3. pH-dependent kinetic data (k_{obs} s^{-1}) for the axial ligation of bromomethyl(aquo)cobaloxime by different ligands at 25°C.

	pH				
Amine	9·5	10·0	10·5	11·0	11·5
Methylamine	0·0005	0·0007	0·0012	0·0031	0·0037
Ethylamine	0·0006	0·0008	0·0013	0·0038	0·0043
Propylamine	0·0007	0·001	0·0015	0·0042	0·0046
Butylamine	0·0008	0·0011	0·0018	0·0046	0·0056
Pentylamine	0·0013	0·002	0·0037	0·0056	0·0068
Hexylamine	0·0015	0·002	0·0041	0·0068	0·0079
Cycloheptylamine	0·0005	0·0006	0·0011	0·0027	0·0035
Cyclohexylamine	0·0004	0·0006	0·001	0·0023	0·0028
Cyclopentylamine	0·0003	0·0004	0·0009	0·0019	0·0022

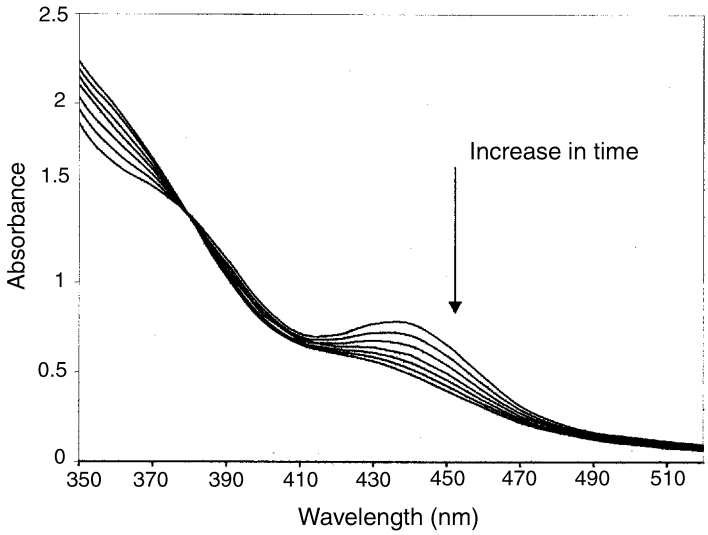

Figure 5. UV-visible scan of kinetics of association of $BrCH_2Co(DH)_2OH_2$ with ethylamine. The spectra were run at time (t) = 0, 3, 6, 9, 12, 15 and 18 min respectively.

related to the fact that the reacting complex is a soft acid while the ligands are hard. The stability of penta-coordinated alkyl cobalt complexes and the evidence that both the dominant soft cobalt (III) complexes $[Co(CN)_5H_2O]^{2-}$ and $[Co(NH_3)_5SO_3]$, undergo SN^1 ligand substitution reactions[37,38] clearly favour this mechanism for the ligation reaction of $BrCH_2Co(DH)_2OH_2$.

To compare the rate constants of linear chain primary amines and cyclo amines for the rate of formation of complex with $BrCH_2Co(DH)_2OH_2$, we have calculated the second-order rate constant k'_{on}. Since this value is also pH-dependent, for better comparison we have calculated k_{on}, the pH-independent second order rate constant. The order of values of k_{on} is as follows: hexylamine > pentylamine > butylamine > propylamine > ethylamine > methylamine > cycloheptylamine > cyclohexylamine > cyclopentylamine.

4. Conclusions

Binding of bromomethyl(aquo)cobaloxime to straight chain primary amines cyclo amines and secondary amines is studied as a function of pH. The strength of binding is explained based on the basicity of the ligand. Straight chain primary amines form more stable complexes than the corresponding cyclo amines. Similarly, the rates of reaction of open chain primary amines are faster than those of cyclo amines. The rate of formation is compared with k_{on}, the pH-independent second-order rate constant.

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