Ligand Substitution Reactions of Aquacobalamin: Evidence for a Dissociative Interchange Mechanism†

Helder M. Marques,* Julia C. Bradley and Linda A. Campbell
Centre for Molecular Design, Department of Chemistry, University of the Witwaterstrand, P.O. Wits, 2050 Johannesburg, South Africa

The kinetics of substitution of bound H_2O in aquacobalamin (vitamin B_{12a}) by hydroxylamine, methyl glycinate, pyridine, 4-methylpyridine, imidazole and histamine (imidazole-4-ethanamine) was investigated as a function of ligand concentration and temperature by stopped-flow spectrophotometry at constant ionic strength (1.0 mol dm^{-3}) and pH. In all six cases the observed pseudo-first-order rate constants, corrected for protonation of the N-donor of the ligand, L, and the ionisation of bound H_2O in B_{12a}, showed saturation at high ligand concentrations. There is a compensating change in the $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ values of the saturating rate constant, k_{sat}, but there is no general isokinetic relationship for the ligands. The dependence of the value of k_{sat}, and, in particular, the dependence of $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ for this rate constant on the entering ligand, L, indicate that the rate-limiting step in the reaction is not unimolecular release of H_2O from B_{12a}. The results are interpreted in terms of a dissociative interchange mechanism with nucleophilic participation of L in the transition state. The compensating effect is explained on the basis of the extent of bond formation between Co and L in the transition state. The steric bulk of L, quantified using molecular mechanics techniques in terms of the cone angle subtended by co-ordinated L at Co^{III}, and molecular volume calculations, appears to play an important role in controlling its rate of reaction with the metal centre.

The mechanism of ligand substitution reactions of cobalt corrinoids has attracted considerable attention,[1-21] not least because the cobalt(III) ion, which is usually kinetically inert, is labilised considerably by the corrin ring (the *cis* labilising effect). We have been particularly interested in the mechanism of substitution of axially co-ordinated H_2O in aquacobalamin (vitamin B_{12a}, here written as dmbzim–Co–H_2O, where dmbzim = 5,6-dimethylbenzimidazole; only the axial ligands are shown and the overall charge is neglected for convenience) by an incoming ligand, L, in aqueous solution. There has been some disagreement concerning the mechanism of these reactions. Some workers have favoured a limiting dissociative (D) mechanism in which the unimolecular dissociation of H_2O from the co-ordination sphere of Co^{III} is rate limiting;[3,5,8,16,17] others have favoured an interchange mechanism (I_d) with participation by the incoming ligand in the transition state;[2,7] yet others[1,6,10] have simply indicated that their results may be interpreted by either mechanism.

Among the evidence offered for a D mechanism is the very modest dependence of the rate constants on the identity of L. We have pointed out, however, that there are compensating changes in $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ for the reaction with a series of primary amines[19,21] and the slope of a plot of $\Delta H^{\ddagger}$ against $\Delta S^{\ddagger}$ happens to be such that, near room temperature, these ligands, at least, react at a similar rate. Measurements of the volumes of activation of the reactions are usually useful in settling questions such as this (and attempts have been made to do so[16,17]). We have recently shown,[20] however, that their interpretation in B_{12a} chemistry is not straightforward and that these measurements fail to distinguish a D and an I_d mechanism.

As expected for a D mechanism, activation enthalpies and entropies for the substitution of H_2O by small ionic ligands such as SCN^-, $S_2O_3^{2-}$, NO_2^- and HSO_3^- are all very similar.[18] However, we have also shown that the transition state for substitution of H_2O by CN^- is enthalpically stabilised by more than 20 kJ mol^{-1} relative to that for substitution by HCN, and that $\Delta S^{\ddagger}$ for the two reactions is −25 and +50 J K^{-1} mol^{-1}, respectively.[14,18] The reaction with SO_3^{2-} has anomalously large $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ values, which we have attributed to the *trans* labilising effect of this ligand in the transition state.[18] The rate of substitution of bound H_2O by a series of imidazoles varies linearly with the basicity of the incoming ligand.[15] We have concluded, therefore, that the underlying mechanism for the ligand substitution reactions of aquacobalamin involves nucleophilic participation of the incoming ligand in the transition state, and is therefore best characterised as an I_d mechanism.

Recently Stochel and van Eldik[17] used pyridine to demonstrate that a plot of observed rate constant against ligand concentration shows saturating behaviour at high (*i.e.* >0.5 mol dm^{-3}) ligand concentrations. If a range of ligands can be found which display similar behaviour then from the values of the saturating rate constants in general and, in particular, from the values of $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ for this rate constant, conclusions might be drawn concerning the underlying mechanism of the process.

For a D mechanism, equations (1) and (2) apply. If k_{-2} is

$$\text{dmbzim–Co–}H_2O \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{dmbzim–Co} + H_2O \quad (1)$$

$$\text{dmbzim–Co} + L \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} \text{dmbzim–Co–L} \quad (2)$$

significant then the observed rate constant, k_{obs}, is related to the microscopic rate constants by equation (3); if not, then equation (3) simplifies to (4). If equation (3) applies a plot of k_{obs} as a

$$k_{obs} = \frac{k_1k_2[L] + k_{-1}k_{-2}}{k_{-1} + k_2[L]} \quad (3)$$

† *Supplementary data available* (No. SUP 56880, 15 pp.): primary kinetic data. See Instructions for Authors, *J. Chem. Soc., Dalton Trans.*, 1992, Issue 1, pp. xx–xxv.

Table 1 Acid dissociation constants, pK_L, for ligands used in this study

Ligand	pK_L (25 °C)	ΔH/kJ mol^{-1}	ΔS/J K^{-1} mol^{-1}	Ref.
Hydroxylamine	6.14	39.9	16	19
Methyl glycinate	7.62	45.6	6	19
Pyridine	5.25	22.6	−26	24
4-Methylpyridine	6.02	26.6	−26	24,25
Imidazole	7.30	38.1	−12	15,24
Histamine	6.41	32.7	−13	15,24

$$k_{obs} = \frac{k_1k_2[L]}{k_{-1} + k_2[L]} \quad (4)$$

function of [L] should be hyperbolic, with a y-axis intercept equal to k_{-2}, an initial slope of k_1k_2/k_{-1}, and curving at high [L] to reach a saturating value which may be termed k_{sat}; for a D mechanism, $k_{sat} = k_1$. If equation (4) is applicable, then a similar curve arises except for the y intercept, which is the origin.

In the case of an I_d mechanism [equations (5) and (6)] the observed rate constant is given by equation (7) if k_{-4} is significant, and equation (8) if not. Equation (7) has exactly the

$$\text{dmbzim–Co–H}_2\text{O} + \text{L} \overset{K}{\rightleftharpoons} \{\text{H}_2\text{O}\cdots\text{dmbzim–Co}\cdots\text{L}\} \quad (5)$$

$$\{\text{H}_2\text{O}\cdots\text{dmbzim–Co}\cdots\text{L}\} \overset{k_4}{\underset{k_{-4}}{\rightleftharpoons}} \text{dmbzim–Co–L} + \text{H}_2\text{O} \quad (6)$$

$$k_{obs} = \frac{k_4K[L]}{1 + K[L]} + k_{-4} \quad (7)$$

$$k_{obs} = \frac{k_4K[L]}{1 + K[L]} \quad (8)$$

same form as (3); the intercept is equal to k_{-4}, the initial slope is $(k_4 + k_{-4})K$, and $k_{sat} = (k_4 + k_{-4})$. If equation (8) is appropriate, then the intercept = 0, the initial slope = k_4K and $k_{sat} = k_4$.

The similar form of equations (3) and (7) [or (4) and (8)] means that a distinction between the two mechanisms is generally not possible. However, if a *series* of ligands can be found which show the hyperbolic behaviour described by these equations then, in principle, a distinction can be made between the two mechanisms. For a D mechanism, k_{sat} corresponds to the rate constant for the unimolecular release of water from the co-ordination sphere of Co^{III}. The value of k_{sat} should be independent of L. It is in particular the independence of $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ on the identity of L which is convincing evidence for a D mechanism. In the case of an I_d mechanism, k_4, which can be obtained from the difference between k_{sat} and the intercept, and its activation parameters, should depend on L.

We report here the results of an investigation of the kinetics of substitution of H_2O in aquacobalamin by a number of N-donor ligands, *viz.* hydroxylamine, methyl glycinate, imidazole, pyridine, 4-methylpyridine and histamine (imidazole-4-ethanamine). In all cases, saturating behaviour with increasing ligand concentration was found. The activation parameters for the saturating rate constants are strongly dependent on L, which is consistent with an I_d mechanism.

Experimental

Hydroxocobalamin (>99% pure, HPLC) was obtained from Roussel. Imidazole (Merck), pyridine (Merck), 4-methylpyridine (Fluka), hydroxylamine hydrochloride (Aldrich), methyl glycinate (glycine methyl ester hydrochloride, Aldrich) and histamine hydrochloride (Merck) were all of the highest purity available and used as received. Water was purified by double distillation in an all-glass still and passage through a Millipore MilliQ system. Preliminary spectroscopic investigations were carried out on a Cary 2300 or 219 UV/VIS spectrophotometer. All reactions were studied under pseudo-first-order conditions at a single pH, halfway between the pK_a of the N-donor of the ligand and the pK_a of co-ordinated H_2O in B_{12a}. The concentrations of B_{12a} were between 50 and 80 μmol dm^{-3}, buffered with phosphate (0.1 mol dm^{-3}) and the total ionic strength was adjusted to 1.00 mol dm^{-3} with KCl. Ligand solutions (between 9 and 15 solutions varying in concentration between *ca.* 0.01 and 1 mol dm^{-3}) were also buffered with phosphate (0.1 mol dm^{-3}) and adjusted to the same pH as that of the B_{12a} solution by addition of HCl or NaOH, as appropriate. The total ionic strength was adjusted to 1.00 mol dm^{-3} with KCl, except for the higher concentrations (>*ca.* 0.5 mol dm^{-3}) of methyl glycinate, hydroxylamine hydrochloride and histamine hydrochloride, where the ionic strength unavoidably exceeded this value. The reactions were usually monitored at 351 nm, the γ-band maximum for aquacobalamin, or 358–362 nm, the γ band for the complex, by mixing equal volumes (0.1 cm^3) of the two solutions using a Hi-Tech Scientific SF-51 stopped-flow spectrometer (cell pathlength 1.00 cm) interfaced through a DAS-50 A/D board with an IBM-type personal computer. The experimentally determined rate constants, k'_{obs}, were found by fitting the absorbance *vs.* time trace to an equation of the form $A_1\exp(-k'_{obs}t) + A_2$, or, where appropriate (see Results), to $A_1\exp(-k'_{1,obs}t) + A_2\exp(-k'_{2,obs}t) + A_3$ using a non-linear least-squares technique employing a Newton–Raphson procedure. The temperature of the system was maintained (±0.1 °C) with a water-circulating bath. The pH of solutions was measured with a Metrohm 605 ion/pH meter and a 6.0210.100 micro combination glass electrode calibrated against standard buffers, all maintained at the appropriate temperature with a water-circulating bath. Data analysis, which is elaborated on below, was by means of either a non-linear least-squares technique employing a Newton–Raphson procedure and Marquardt's algorithm, or by standard linear least-squares methods.[22]

Results

The dependence on pH of the experimentally determined pseudo-first-order rate constants, k'_{obs}, for the ligand substitution reactions of B_{12a} is well established.[7,14,20,21,23] Hydroxocobalamin is inert to substitution, and protonated amines will not co-ordinate. The reactions were therefore studied at a single pH, between 6.5 and 7.5, where the observed reaction rate is at a maximum (details in SUP 56880). For equations (3), (4), (7) and (8) to hold, k'_{obs} must be converted into a pH-independent rate constant, k_{obs}, using equation (9). In equation (9), pK_{Co} is

$$k_{obs} = k'_{obs}(1 + [H^+]/K_L)(1 + K_{Co}/[H^+]) \quad (9)$$

the temperature-dependent acid dissociation constant for co-ordinated H_2O in B_{12a} (ΔH = 28.6 ± 0.3 kJ mol^{-1}, ΔS = −59.0 ± 1.2 J K^{-1} mol^{-1} at 1.00 mol dm^{-3} ionic strength[14]) and pK_L the temperature-dependent acid dissociation constant for the donor N atom of the ligand. The values of pK_L used are given in Table 1.

The kinetics of the reaction of B_{12a} with pyridine as a function of ligand concentration, temperature and pressure at 0.5 mol dm^{-3} ionic strength has been reported recently.[17] This reaction was reinvestigated to verify the results. We have previously reported on the kinetics of substitution by imidazole[15] (but only at 25 °C), hydroxylamine[19] and methyl glycinate,[19,21] but only relatively low concentrations (<0.2 mol dm^{-3}) of the ligands were used so that any curvature occurring at higher concentrations would have been missed. The values obtained

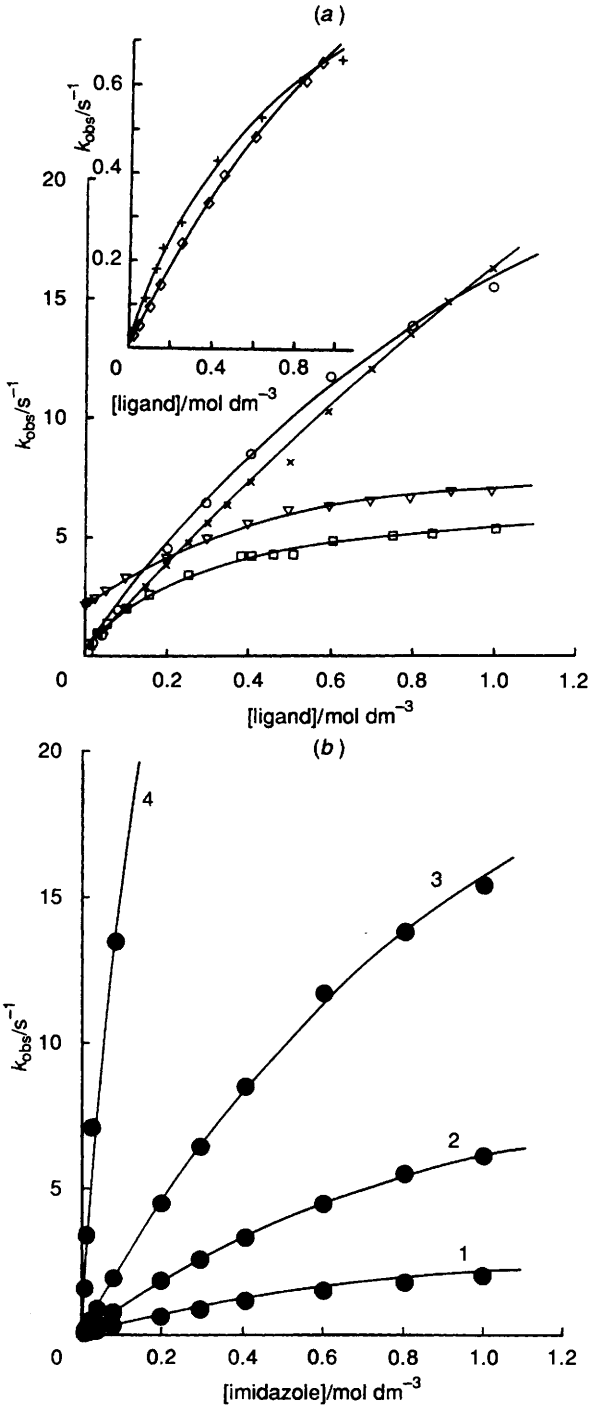


Fig. 1 (*a*) Dependence of k_{obs} on ligand concentration for hydroxylamine (×), imidazole (○), pyridine (▽), 4-methylpyridine (□), (insert) methyl glycinate (+), and histamine (◇). All data at 25 °C except for histamine (24.2 °C) and methyl glycinate (25.2 °C). (*b*) Dependence of k_{obs} on [imidazole] and temperature for the reaction of imidazole with aquacobalamin at 5.7 (1), 15.8 (2), 25.0 (3) and 45.0 °C (4)

for k'_{obs} for the six ligands studied (hydroxylamine, methyl glycinate, pyridine, 4-methylpyridine, imidazole and histamine), together with the pH-corrected rate constants, k_{obs}, are available as SUP 56880. Values of k_{obs} were plotted as a function of ligand concentration; in all cases, significant curvature was seen (Fig. 1).

For hydroxylamine, methyl glycinate, histamine and imidazole the *y*-axis intercepts were within two standard deviations of the origin, which means that the reverse rate constants [k_{-2} in equation (3) or k_{-4} in (7)] are very small. For pyridine (in agreement with the observations of Stochel and van Eldik [17]) and for 4-methylpyridine the rate constant for dissociation of the ligand from Co^{III} is significant. Although significant curvature is seen in all plots, it is clear that complete saturation is not attainable in the ligand concentration range studied so that reliable values for the saturating rate constant are difficult to obtain. Higher ligand concentrations could not generally be used. In the case of pyridine, 4-methylpyridine and histamine a large initial signal change occurs on first mixing, before the onset of the exponential absorbance change which can be attributed to the reaction. The size of this initial signal increased with ligand concentration and we suspect that it is due to the mixing of solutions of very different refractive index. [No such effects were noted with comparable concentrations of solutions of ionic salts, such as iron(III) nitrate and potassium thiocyanate, for example.] The inevitable consequence was that significant fractions of the absorbance change due to the reaction were lost at the higher ligand concentrations. In the case of methyl glycinate, a 2 mol dm^{-3} solution (which, on mixing, leads to a solution 1 mol dm^{-3} in ligand) is close to the solubility limit.

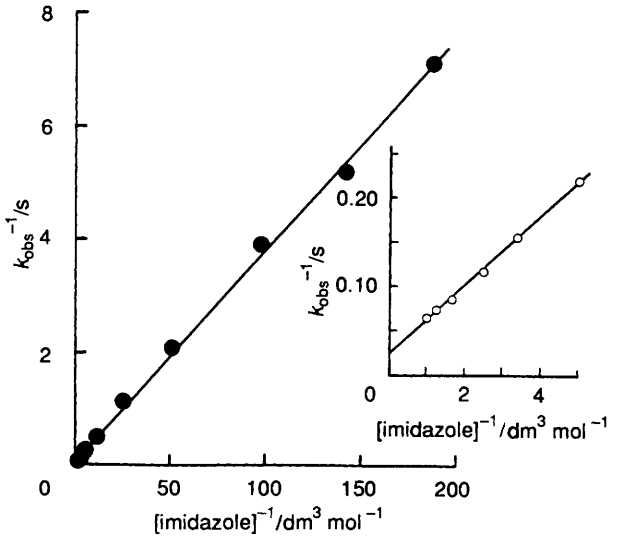


Fig. 2 Plots of k_{obs}^{-1} against [imidazole]$^{-1}$ for the reaction of imidazole with aquacobalamin at 25 °C. The value of k_4 was determined from the intercept (insert) using data for the six most concentrated ligand solutions used, and K from the slope using the full data set

Alternative ways of obtaining estimates for k_{sat} were sought. It is evident from Fig. 1 that the values of the saturating rate constants vary significantly which suggests, as discussed in the Introduction, that an I_d mechanism [equations (5) and (6)] is more appropriate than the D mechanism of equations (1) and (2). We therefore fitted the experimental data by equation (7) or (8), as appropriate.

By rearranging terms and inverting, equations (7) and (8) lead to (10) and (11). Plots of $1(k_{obs} - k_{-4})$ against 1/[L]

$$\frac{1}{k_{obs} - k_{-4}} = \frac{1}{k_4 K[L]} + \frac{1}{k_4} \quad (10)$$

$$\frac{1}{k_{obs}} = \frac{1}{k_4 K[L]} + \frac{1}{k_4} \quad (11)$$

[where k_{-4} is available from equation (7)] or $1/k_{obs}$ against 1/[L] should be linear where k_4 can be obtained from the intercept and K from the ratio of the intercept and slope. One of the problems associated with reciprocal plots such as this is the excessive weighting which the data points corresponding to low ligand concentrations will carry. Even small errors in these values will lead to a significant skewing of the fit parameters, and especially the intercept. For this reason, only the last six or seven points of each data set (corresponding to the higher ligand

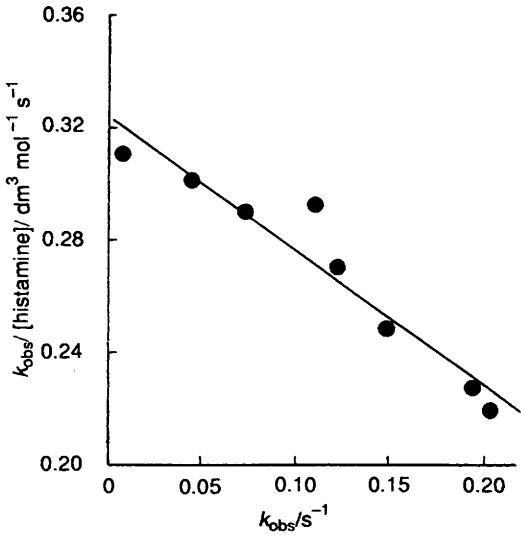


Fig. 3 Plot of k_{obs}/[histamine] against k_{obs} for the reaction of histamine with aquacobalamin at 15 °C

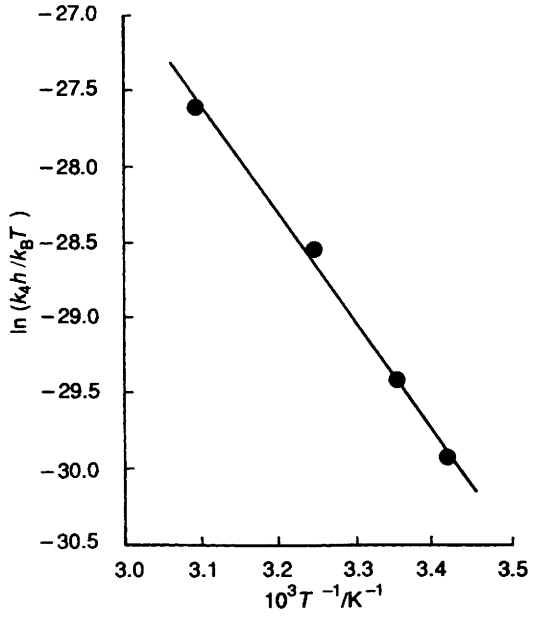


Fig. 4 Temperature dependence of k_4 for the reaction of methyl glycinate with aquacobalamin °C

concentrations) were used to obtain values of k_4; however, the complete data set was used for obtaining the values of K. Fig. 2 shows an example of the fits obtained using this method.

Equations (7) and (8) can also be manipulated to yield the linear equations (12) and (13). A plot of $(k_{obs} - k_{-4})/[L]$ against

$$\frac{k_{obs} - k_{-4}}{[L]} = -k_{obs}K + (k_4 + k_{-4})K \qquad (12)$$

$$\frac{k_{obs}}{[L]} = -k_{obs}K + k_4K \qquad (13)$$

k_{obs} gives K from the slope and the sum $k_4 + k_{-4}$ from the ratio of the intercept and slope. If k_{-4} is insignificant, then a plot of $k_{obs}/[L]$ against k_{obs} will yield K and k_4, respectively. An example of the fits obtained (using the full data set) is shown in Fig. 3. The values of k_4, k_{-4} and K obtained from these various plots, and their averages, are listed in Table 2.

At 5.4 °C the plot of k_{obs} against [hydroxylamine] is a straight line, *i.e.* curvature occurs at higher ligand concentrations than used in this study. The slope of this line corresponds to k_4K. The K values obtained at 15.0, 25.0 and 36.2 °C were plotted against T^{-1}. From the slope of the resulting straight line $\Delta H = 45 \pm 7$ kJ mol^{-1} and from the intercept $\Delta S = 139 \pm 24$ J K^{-1} mol^{-1}. Hence K at 5.4 °C is estimated to be 0.066 dm^3 mol^{-1} and therefore $k_4 = 35$ s^{-1}.

From the temperature dependence of k_4 and (where applicable) k_{-4} the activation parameters of the reactions were determined by plotting ln (k_ih/k_BT), where h and k_B are the Planck and Boltzmann constants, respectively, and $i = 4$ or -4, against T^{-1}. Fig. 4 is an example of such a plot and the activation parameters determined are listed in Table 3. There is considerable uncertainty in the values of the pre-equilibrium constant K (although the data for hydroxylamine, perhaps fortuitously, yielded a reasonable straight line); it was assumed that a plot of ln K against T^{-1} was linear and ΔH° and ΔS° values were determined from the slopes and intercepts. These values are also listed in Table 3.

Discussion

The ligand substitution reactions of the cobalamins have generally been assumed to be 'simple', in that they conform to the classical schemes of ligand substitution reactions of transition-metal ions, as given in equations (1) and (2), or (5) and (6).[4,13] When re-examining the kinetics of the reaction of B_{12a} with pyridine at 362.5 nm we found that a single exponential fit to the absorbance *vs.* time traces gave rise to systematic deviation of the residuals, and the traces could best be fitted using a two-exponential expression, indicating that at least two kinetically distinguishable processes are occurring. The faster phase was about 3 times faster than the slower phase and usually accounted for about 15% of the total observed signal change. When the reaction was monitored at 351 nm using the same solutions only a single phase was observed and the rate corresponded very well to the slow phase observed at 362.5 nm. No analogous effects were observed with the other ligands investigated in this study. This does not necessarily mean the presence of only one phase; a single exponential change in absorbance merely requires that the rate of the two phases not be very different. The origin of the fast phase is obscure. It is mathematically impossible to distinguish parallel and consecutive processes;[26] hence we could have been observing the formation of some unstable intermediate which then, in a subsequent slower step, rearranged to the final equilibrium product, or a parallel process where the approach of the ligand to the metal ion followed at least two kinetically distinguishable trajectories. Why this process is observable at one wavelength but not at another is not clear, and clearly requires further investigation. It must therefore be accepted that the ligand substitution reactions of B_{12a} may be more complex than we and others have assumed, and that the reaction schemes as given in equations (1) and (2), and (5) and (6), may at best be approximations of the events occurring at the molecular level. With this caveat in mind, it is nevertheless possible to draw some conclusions about the mechanism of these reactions from the present study.

There are problems associated with the interpretation of kinetic data on asymmetrically substituted imidazoles. Species such as histamine exist in solution as a mixture of the 4- and the 5-substituted tautomers [equation (14)], governed by a tautomerism equilibrium constant, K_T [equation (15)].[27] We have previously shown,[15] by analogy with 2-methylimidazole, that the rate of reaction of the 4 tautomer of an asymmetrically substituted imidazole is insignificant compared to that of the 5 tautomer; the effective concentration of the reactive species in solution should therefore be adjusted by a factor of $1/(1 + K_T)$. Monocationic histamine has been shown to exist in solution with the 4 tautomer as the favoured species,[28] and we have estimated $K_T = 5.2$ dm^3 mol^{-1}.[15] If this complication is taken into account, then K will increase from 0.5 to 3.1 dm^3 mol^{-1} at

Table 2 Rate and equilibrium constants for the co-ordination of the ligand, L, by aquacobalamin

Ligand	Parameter	T^a/°C	Determined using equation	Value	Average[b]
Hydroxylamine	K/dm^3 mol^{-1}	5.4	See text	0.066	0.066
		15.0	(8)	0.14 ± 0.01	0.14 ± 0.01
			(11)	0.13 ± 0.02	
			(13)	0.14 ± 0.01	
		25.0	(8)	0.22 ± 0.01	0.25 ± 0.03
			(11)	0.28 ± 0.01	
			(13)	0.24 ± 0.01	
		36.2	(8)	0.71 ± 0.04	0.62 ± 0.09
			(11)	0.54 ± 0.03	
			(13)	0.62 ± 0.04	
	k_4/s^{-1}	5.4	See text	35	35
		15.0	(8)	51 ± 5	53 ± 4
			(11)	58 ± 10	
			(13)	50 ± 6	
		25.0	(8)	89 ± 3	87 ± 3
			(11)	88 ± 3	
			(13)	84 ± 4	
		36.2	(8)	99 ± 5	101 ± 8
			(11)	94 ± 7	
			(13)	109 ± 5	
Methyl glycinate	K/dm^3 mol^{-1}	19.7	(8)	1.6 ± 0.2	2.0 ± 0.4
			(11)	2.4 ± 0.2	
			(13)	1.9 ± 0.2	
		25.2	(8)	1.6 ± 0.1	1.7 ± 0.2
			(11)	1.9 ± 0.2	
			(13)	1.7 ± 0.2	
		35.1	(8)	1.7 ± 0.2	1.8 ± 0.2
			(11)	2.0 ± 0.3	
			(13)	1.7 ± 0.2	
		50.1	(8)	2.7 ± 0.2	2.9 ± 0.3
			(11)	3.3 ± 0.2	
			(13)	2.8 ± 0.2	
	k_4/s^{-1}	19.7	(8)	0.64 ± 0.04	0.61 ± 0.02
			(11)	0.59 ± 0.06	
			(13)	0.59 ± 0.09	
		25.2	(8)	1.16 ± 0.03	1.03 ± 0.04
			(11)	0.99 ± 0.09	
			(13)	1.03 ± 0.08	
		35.1	(8)	2.7 ± 0.2	2.6 ± 0.2
			(11)	2.3 ± 0.2	
			(13)	2.7 ± 0.2	
		50.1	(8)	7.0 ± 0.3	6.9 ± 0.3
			(11)	6.6 ± 0.4	
			(13)	7.0 ± 0.9	
Pyridine[c]	K/dm^3 mol^{-1}	5.0	(7)	2.5 ± 0.3	2.5 ± 0.1
			(10)	(3.3 ± 0.5)	
			(12)	2.4 ± 0.2	
		15.0	(7)	2.2 ± 0.3	2.3 ± 0.3
			(10)	2.5 ± 0.3	
			(12)	2.0 ± 0.1	
		25.0	(7)	2.5 ± 0.2	2.4 ± 0.1
			(10)	2.4 ± 0.2	
			(12)	2.4 ± 0.1	
		35.0	(7)	2.9 ± 0.4	3.5 ± 0.7
			(10)	(7.2 ± 2.1)	
			(12)	4.0 ± 0.7	
	k_4/s^{-1}	5.0	(7)	0.61 ± 0.02	0.63 ± 0.03
			(10)	0.66 ± 0.03	
			(12)	0.62 ± 0.08	
		15.0	(7)	2.4 ± 0.1	2.5 ± 0.1
			(10)	2.6 ± 0.1	
			(12)	2.5 ± 0.3	
		25.0	(7)	7.0 ± 0.2	7.1 ± 0.1
			(10)	7.1 ± 0.3	
			(12)	7.2 ± 0.5	
		35.0	(7)	20.2 ± 0.9	21.2 ± 1.4
			(10)	22.2 ± 1.3	
			(12)	(18.2 ± 7.3)	
		5.0	(7)		0.115 ± 0.006
		15.0	(7)		0.52 ± 0.02
		25.0	(7)		1.99 ± 0.05
		35.0	(7)		6.9 ± 0.3

Table 2 (*continued*)

Ligand	Parameter	T^a/°C	Determined using equation	Value	Average[b]
d	K/dm^3 mol^{-1}	5.0	(7)		4.3 ± 0.6
		15.0	(7)		3.0 ± 0.3
		25.0	(7)		2.4 ± 0.4
		35.0	(7)		2.1 ± 0.5
	k_4/s^{-1}	5.0	(7)		0.65 ± 0.02
		15.0	(7)		2.58 ± 0.08
		25.0	(7)		7.1 ± 0.4
		35.0	(7)		14.2 ± 1.2
	k_{-4}/s^{-1}	5.0	(7)		0.10 ± 0.01
		15.0	(7)		0.41 ± 0.03
		25.0	(7)		2.4 ± 0.4
		35.0	(7)		7.6 ± 0.3
4-Methylpyridine	K/dm^3 mol^{-1}	5.7	(7)	2.9 ± 0.2	2.8 ± 0.1
			(10)	2.8 ± 0.3	
			(12)	2.6 ± 0.1	
		15.3	(7)	2.9 ± 0.2	2.9 ± 0.1
			(10)	2.9 ± 0.3	
			(12)	2.8 ± 0.1	
		25.0	(7)	3.4 ± 0.3	3.3 ± 0.1
			(10)	3.4 ± 0.2	
			(12)	3.2 ± 0.1	
		35.0	(7)	3.9 ± 0.3	3.6 ± 0.3
			(10)	3.3 ± 0.2	
			(12)	3.7 ± 0.1	
	k_4/s^{-1}	5.7	(7)	0.62 ± 0.01	0.61 ± 0.02
			(10)	0.59 ± 0.01	
			(12)	0.64 ± 0.05	
		15.3	(7)	2.24 ± 0.04	2.24 ± 0.04
			(10)	2.2 ± 0.2	
			(12)	2.3 ± 0.1	
		25.0	(7)	6.4 ± 0.2	6.4 ± 0.2
			(10)	6.2 ± 0.2	
			(12)	6.5 ± 0.6	
		35.0	(7)	16.0 ± 0.3	15.9 ± 0.5
			(10)	15.3 ± 0.4	
			(12)	16.3 ± 1.1	
	k_{-4}/s^{-1}	5.0	(7)		0.025 ± 0.005
		15.0			0.11 ± 0.02
		25.0			0.44 ± 0.08
		35.0			1.46 ± 0.2
Imidazole	K/dm^3 mol^{-1}	5.7	(8)	0.92 ± 0.05	0.93 ± 0.01
			(11)	(1.4 ± 0.7)	
			(13)	0.93 ± 0.09	
		15.8	(8)	0.73 ± 0.03	0.63 ± 0.04
			(11)	0.65 ± 0.53	
			(13)	0.67 ± 0.05	
		25.0	(8)	0.60 ± 0.06	0.65 ± 0.07
			(11)	0.61 ± 0.75	
			(13)	0.73 ± 0.06	
		45.0	(8)	0.38 ± 0.05	0.42 ± 0.14
			(11)	0.3 ± 0.2	
			(13)	0.58 ± 0.09	
	k_4/s^{-1}	5.7	(8)	4.3 ± 0.1	4.5 ± 0.3
			(11)	4.8 ± 0.2	
			(13)	4.3 ± 0.1	
		15.8	(8)	14.7 ± 0.5	14.9 ± 0.6
			(11)	14.5 ± 0.5	
			(13)	15.6 ± 0.4	
		25.0	(8)	44.2 ± 3.5	43.3 ± 0.9
			(11)	42.4 ± 5.7	
			(13)	(37.0 ± 1.9)	
		45.0	(8)	219 ± 12	209 ± 9
			(11)	201 ± 15	
			(13)	207 ± 15	
Histamine	K/dm^3 mol^{-1}	9.2	(8)	1.7 ± 0.2	1.0 ± 0.6
			(11)	0.4 ± 0.4	
			(13)	0.8 ± 0.2	
		15.6	(8)	0.60 ± 0.07	0.65 ± 0.10
			(11)	0.58 ± 0.12	
			(13)	0.77 ± 0.06	
		20.0	(8)	0.49 ± 0.06	0.59 ± 0.09
			(11)	0.62 ± 0.09	
			(13)	0.66 ± 0.08	

Table 2 (*continued*)

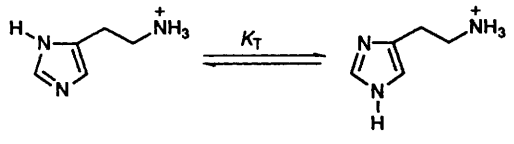
Ligand	Parameter	T^a/°C	Determined using equation	Value	Average[b]
Histamine	K/dm^3 mol^{-1}	24.2	(8)	0.59 ± 0.03	0.49 ± 0.15
			(11)	0.32 ± 0.18	
			(13)	0.56 ± 0.07	
		35.0	(8)	0.38 ± 0.02	0.33 ± 0.04
			(11)	0.31 ± 0.10	
			(13)	0.30 ± 0.04	
	k/s^{-1}	9.2	(8)	0.16 ± 0.01	0.19 ± 0.05
			(11)	0.16 ± 0.01	
			(13)	0.25 ± 0.12	
		15.6	(8)	0.54 ± 0.04	0.58 ± 0.04
			(11)	0.63 ± 0.09	
			(13)	0.58 ± 0.11	
		20.0	(8)	1.1 ± 0.1	1.2 ± 0.1
			(11)	1.2 ± 0.4	
			(13)	1.2 ± 0.3	
		24.2	(8)	1.85 ± 0.07	2.0 ± 0.2
			(11)	2.2 ± 0.2	
			(13)	1.9 ± 0.3	
		35.0	(8)	8.63 ± 0.05	8.5 ± 0.7
			(11)	9.1 ± 0.4	
			(13)	7.8 ± 0.4	

[a] ±0.1 °C. [b] Values in parentheses were omitted. [c] Monitoring wavelength = 351 nm. [d] Monitoring wavelength = 362.5 nm.

Table 3 Rate and thermodynamic parameters for the reaction of aquacobalamin with the ligand, L

Ligand	Parameter	$\Delta H^{\ddagger}$/kJ mol^{-1}	$\Delta S^{\ddagger}$/J K^{-1} mol^{-1}	ΔH°/kJ mol^{-1}	ΔS°/J K^{-1} mol^{-1}	k_4 (25 °C)[a]/s^{-1}	K (25 °C)[b]/ dm^3 mol^{-1}
Hydroxylamine	k_4	23 ± 4	−131 ± 12			83	0.2
	K			45 ± 7	139 ± 24		
Methyl glycinate	k_4	60 ± 3	−43 ± 10			1.1	2.2
	K			10 ± 7	40 ± 22		
Pyridine	k_4	80 ± 2	41 ± 1			8.2	3.1
	k_{-4}	95 ± 1	79 ± 3				
	K			7 ± 5	33 ± 19		
4-Methylpyridine	k_4	78 ± 3	32 ± 9			6.2	3.1
	k_{-4}	99 ± 1	81 ± 4				
	K			6 ± 1	31 ± 4		
Imidazole	k_4	70 ± 4	19 ± 12			33	0.6
	K			−14 ± 3	−51 ± 9		
Histamine	k_4	101 ± 4	101 ± 14			2.3	0.5
	K			−30 ± 2	−106 ± 8		

[a] Calculated using the $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ values in columns 3 and 4. [b] Calculated using the values of ΔH° and ΔS° in columns 5 and 6.



5 tautomer 4 tautomer (14)

$$K_T = [4\text{ tautomer}] / [5\text{ tautomer}] \quad (15)$$

25 °C, although there will be no effect on the value of k_{sat}. The problem of the tautomerism of histamine is therefore of no consequence if the focus of the discussion is on the values of the saturating rate constant for the various ligands being examined. (Since the value of K_T is at best an approximation, it should be noted that the data of Tables 2 and 3 have not been corrected for the tautomerism of histamine.)

For convenience the saturating rate constants calculated for the six ligands studied are compared at 25 °C in Table 3. From these results (and see Fig. 1) a conclusion can be reached concerning the main issue addressed in this study. It is evident that there is a dependence of k_{sat} on L with rates ranging between 1.1 s^{-1} for methyl glycinate and 83 s^{-1} for hydroxylamine at 25 °C. More important is the marked dependence of $\Delta H^{\ddagger}$ (which ranges from 23 for hydroxylamine to 101 kJ mol^{-1} for histamine) and $\Delta S^{\ddagger}$ (ranging from −131 for hydroxylamine to 101 J K^{-1} mol^{-1} for histamine). It is therefore concluded that k_{sat} is unlikely to correspond to k_1, but rather to k_4 (or $k_4 + k_{-4}$ if k_{-4} is significant). Consequently the mechanism is best characterised as I_d, allowing for nucleophilic participation by the incoming ligand in the transition state.

A plot of $\Delta H^{\ddagger}$ against $\Delta S^{\ddagger}$ for k_4 is a straight line (Fig. 5). A similar correlation has previously been shown between these two quantities for the reaction of B_{12a} with primary amines [19,21] and it would appear that a compensation effect between the entropy and enthalpy of activation may be a general feature of the ligand substitution reactions of B_{12a}. Although there may be other interpretations possible for the compensation effect, we believe that these parameters are a measure of the extent of involvement of the incoming ligand in the transiton state of the reaction. Considerable bond formation between L and Co will compensate for bond breaking between Co and H_2O, $\Delta H^{\ddagger}$ is small and, consequently, $\Delta S^{\ddagger}$ is large and negative due to the loss of freedom of the ligand; conversely, a large value of $\Delta H^{\ddagger}$ entails $\Delta S^{\ddagger}$ being large and positive as bond breaking between Co and O is the dominant process in the transition state. This compensation effect may mask the dependence of k_{sat} on L and

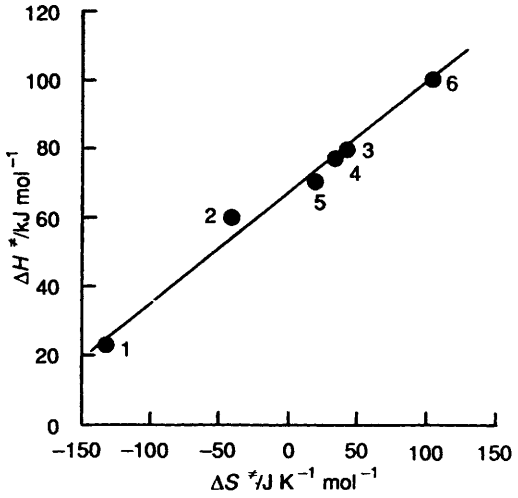


Fig. 5 Relationship between $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ for k_4: 1, NH_2OH; 2, $NH_2CH_2CO_2Me$; 3, pyridine; 4, 4-methylpyridine; 5, imidazole; 6, histamine

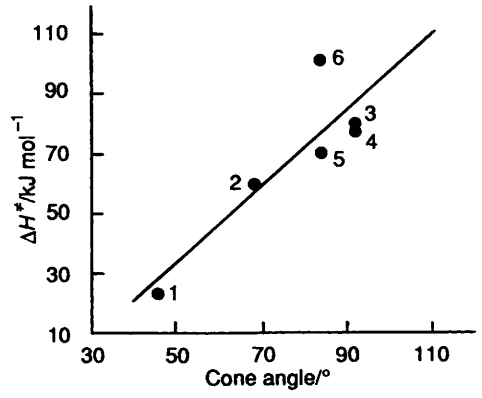


Fig. 6 Relationship between $\Delta H^{\ddagger}$ for k_4 and the cone angle subtended by the ligand at the metal ion as calculated by molecular mechanics techniques. Numbers as in Fig. 5

Table 4 Calculated ligand steric parameters

Ligand	Cone angle[a]/°	Molecular volume[b]/Å^3
Hydroxylamine	45.2	29.7
Methyl glycinate	68.3	91.0
Pyridine	91.9	87.7
4-Methylpyridine	91.9	104.7
Imidazole	82.9	78.7
Histamine	83.5[c]	126.9

[a] By molecular mechanics at an arbitrary Co–N bond length of 1.960 Å. [b] By QCPE 509. [c] The value of the cone angle depends on the orientation of the side-chain relative to the metal ion. At its furthest orientation (which is the most likely conformation given the steric crowding around the metal centre) the cone angle [(C^2)H–Co–H(C^4)] is 83.5°. At the closest approach of the side-chain to the metal ion, the maximum cone angle of 87.5° is between (C^2)H of the ring, the metal ion, and a H atom of the NH_3^+ group of the side-chain.

emphasises our previous comments[18–21] that the apparent insensitivity of rate constants on ligand identity cannot be used as evidence for a D mechanism. At the very least, the independence of $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ on the indentity of L will have to be demonstrated for a convincing case to be made for a D mechanism.

It should be appreciated that there is no general isokinetic relationship between values of k_4 for the ligands studied since plots of ln k_4 against T^{-1} do not intersect at a common point (not shown). Although no generally accepted explanation for

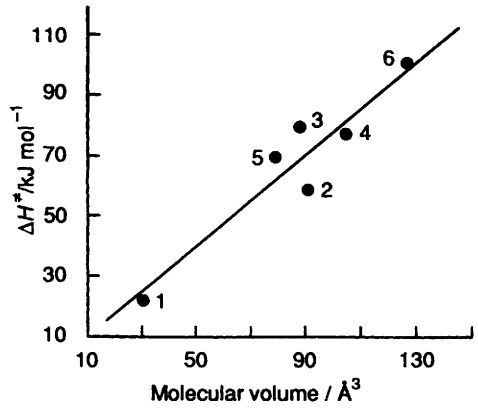


Fig. 7 Relationship between $\Delta H^{\ddagger}$ for k_4 and molecular volume. Numbers as in Fig. 5

the origin of isokinetic relationships appears yet to have been advanced[29] they have been taken as indicative of the operation of a single reaction mechanism.[30–32] The absence of such a relationship may be a further indication of the complexity of these apparently simple reactions.

The results of Table 3 show that there is no particular correlation between, for example, ligand basicity (pK_L, Table 1) and either K or k_4. The six ligands investigated here belong to three very distinct classes of N-donors (primary amines, imidazoles, pyridines). To attempt to arrive at any firm conclusion concerning electronic effects with the very limited data available is unlikely to be productive, and such questions will have to await the availability of a much larger set of data based on structurally similar ligands.

Two approaches were used in attempting to assess whether steric factors are of importance in controlling reaction rates of B_{12a} with these ligands. Our first approach was to utilise a molecular mechanics technique using the program ALCHEMY II.[33] We built molecular fragments consisting of the various ligands studied bonded to Co^{III} at an (arbitrary) equilibrium bond length of 1.960 Å, kept at this value by using a large (1700 kJ mol^{-1} Å^{-2}) bond stretching force constant. (The bond length to the N atom of the sterically hindered axial dmbzim ligand in adenosylcobalamin is 2.25 Å,[34] 2.19 Å in methylcobalamin[35] and 1.97–2.06 Å in cyanocobalamin;[36,37] it is therefore likely to be shorter for the relatively unhindered bases investigated here. The bond length chosen is not important for the purpose of this study provided it is kept constant.) The force-field parameters built into the program were used to find an energy-minimised structure of each ligand–metal pair. We then measured the maximum cone angle subtended at the metal ion by the co-ordinated ligand [for example, in imidazole this is the (C^2)H–Co–H(C^4) angle]. The results obtained are listed in Table 4. In the second approach the program QCPE 509[38] was used to calculate the molecular volumes of the ligands themselves; these values are also listed in Table 4.

There is a reasonable linear relationship between $\Delta H^{\ddagger}$ and cone angle (Fig. 6). Since $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ are correlated (Fig. 5), there is an analogous correlation between $\Delta S^{\ddagger}$ and cone angle. There is also an apparently linear correlation between $\Delta H^{\ddagger}$ and the molecular volume of the incoming ligand (Fig. 7); again, given the relationship of Fig. 5, $\Delta S^{\ddagger}$ correlates approximately linearly with the molecular volume.

Factors such as ligand basicity and the interaction of functional groups on the ligand with the acetamide side-chains of the corrin ring[19,39,40] may, and indeed are likely to, play a role in determining the rate at which a ligand will react at the metal centre. Further, in the case of histamine, the side-chain amino group is positively charged (pK_a = 9.96[15]) at the pH at which the reaction was studied. There is likely to be some coulombic repulsion between the ligand and the net 2+ charge

at the metal centre (+3 from Co and −1 from the corrin N-donor set, assuming the phosphate is too far away, *ca.* 10 Å, to be of importance) in the transition state, and this may partly explain the anomalous position of histamine in Fig. 6 (although there appears to be no such anomaly in Fig. 7). The relationships of Figs. 6 and 7 would suggest that steric factors are of considerable importance. The β face of the corrin ring is sterically crowded[41] with the b and c acetamide side-chains of rings A and B, respectively, and the methyl groups at positions 12 and 17 on the C and D rings, respectively, placing four steric pickets in the pathway of a ligand approaching the metal centre. We propose that steric interactions between these corrin ring substituents and the incoming ligand explain, at least in part, the relationships of Figs. 6 and 7.

It is concluded that, at least for this series of ligands, the dependence of the saturating rate constant and, in particular, the activation parameters on the incoming ligand allows the mechanism of the ligand substitution reactions of vitamin B_{12a} to be characterised as I_d, although it has to be accepted that the reactions may not be as simple as the classical I_d mechanism allows for. The extent of nucleophilic participation of the ligand in the transition state leads to a compensating change in $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$, and the steric bulk of the ligand appears to be an important feature controlling the extent of its bonding to the metal ion in the transition state.

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