Ligand Substitution Reactions of Aquocobalamin. Reactions with Primary Amines

Helder M. Marques
Centre for Molecular Design, Department of Chemistry, University of the Witwatersrand, P.O. Wits, 2050 Johannesburg, South Africa

Enthalpies and entropies of activation were determined from the temperature variation of the spectrophotometrically determined pH-independent rate constants for the reaction of nine neutral primary amines with aquocobalamin (vitamin B_{12a}) in aqueous solution (ionic strength, $I = 1.00$ mol dm^{-3}). There are compensating changes in $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ for this series of ligands, with $\Delta H^{\ddagger}$ decreasing from *ca.* 81 to 58 kJ mol^{-1} as $\Delta S^{\ddagger}$ decreases in parallel from *ca.* 46 to −46 J K^{-1} mol^{-1}. There is, however, no apparent isokinetic relationship for the series. Plots of $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ against the ligand pK_a reveal that the ligands fall into two distinct classes; for a given pK_a value, ligands in the first class [NH_3, $NH_2(CH_2)_3OH$, NH_2Me and NH_2Pr] have significantly larger $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ values than those in the second class [NH_2OMe, NH_2OH, $NH_2CH_2CO_2Me$, $NH_2CH_2CH(OH)CH_2OH$ and $NH_2(CH_2)_2OH$]. This is ascribed to ligands in the second class interacting by hydrogen bonding with the acetamide side-chains of the corrin ring and so having their amino groups favourably oriented for interactions with the metal ion.

Our studies of the kinetics of the ligand substitution reactions of aquocobalamin † (vitamin B_{12a})[1–3] in which H_2O, co-ordinated to Co^{III} in the upper or β axial co-ordination site, is displaced by an incoming ligand, L [equation (1); dmbzim = 5,6-dimethyl-

$$\text{dmbzim–Co–}H_2O + L \underset{k_r}{\overset{k_{II}}{\rightleftharpoons}} \text{dmbzim–Co–L} + H_2O \quad (1)$$

benzimidazole, the α axial ligand in the cobalamins], were prompted by our investigations with L = cyanide.[1] We showed that both CN^- and HCN act as nucleophiles towards Co^{III} with (pH-independent) second-order rate constants of 250 and 82 dm^3 mol^{-1} s^{-1}, respectively, *i.e.*, CN^- reacts only about three times faster than HCN; however, $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ values for the reaction wtih CN^- (51.9 kJ mol^{-1}, −25 J K^{-1} mol^{-1}) are significantly smaller than those for the reaction with HCN (77.0 kJ mol^{-1}, 50 J K^{-1} mol^{-1}) and the similarity of the rate constants at 25 °C is merely due to a compensating change in the two activation parameters. It generally has been accepted,[4,5] based largely upon the observation that the rate of substitution of H_2O by various small anionic ligands varies only by about two orders of magnitude whereas stability constants vary by over 11 orders of magnitude, that the stoichiometric mechanism of the substitution reaction is purely dissociative (D). However, studies invariably have been confined to around 25 °C, and it is conceivable that at this particular temperature the rate constants are *coincidently* very similar although the activation parameters may be very different.[6] Further information concerning the temperature dependence of rate constants is clearly required.

It has been shown[2] for a series of imidazole derivatives that once complications due to ligand tautomerism are taken into account, the rate of substitution of H_2O in B_{12a} increases linearly with the pK_a of the incoming ligand. Activation parameters for substitution of H_2O by a series of small anionic and neutral ligands (CN^-, HCN, N_3^-, HN_3, SCN^-, $S_2O_3^{2-}$, NO_2^-, SO_3^{2-} or HSO_3^-)[3] have been measured and it was demonstrated that although for most of these ligands $\Delta H^{\ddagger}$ (63–70 kJ mol^{-1}) and $\Delta S^{\ddagger}$ (20–28 J K^{-1} mol^{-1}) are very similar, CN^- has significantly smaller activation parameters (see above), which, since it forms a very stable complex with cobalamin (log K = 14.1),[7] was ascribed to significant Co–C bond formation in the transition state; and that the reaction with SO_3^{2-} has a large $\Delta H^{\ddagger}$ (79.9 kJ mol^{-1}) and larger than average $\Delta S^{\ddagger}$ (33 J K^{-1} mol^{-1}), and attributed this to the strong *trans* labilising effect of this ligand causing substantial *trans* Co–N(dmbzim) bond breaking in the transition state. These results prompted us to advance the suggestion that, at least in some cases (imidazoles, CN^-, SO_3^{2-}), there may be significant nucleophilic participation of the incoming ligand in the transition state of the substitution reactions of B_{12a}. This provided experimental evidence in support of the analysis of Reenstra and Jencks[8] who contended that a purely D mechanism in a co-ordinating solvent such as water is highly improbable and that the ligand substitution reactions of B_{12a} proceed through a dissociative interchange mechanism (I_d). The characterisation of the reaction as I_d has not been universally accepted. For example, values of $\Delta V^{\ddagger}$ for substitution of H_2O by cyanoferrates and N_3^- are positive (7–16 cm^3 mol^{-1}) and have been explained by recourse to a limiting D mechanism.[9]

To provide further information concerning the ligand substitution reactions of B_{12a}, activation parameters ($\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$) have been determined from the temperature dependence of the rate constants for a series of primary neutral amines. These were chosen because (*i*) they form reasonably stable complexes with cobalamin (log $K \geqslant 4.5$);[4,10–12] (*ii*) ligands with a wide range of pK_a values are available; (*iii*) significant spectroscopic changes occur in their co-ordination so that the reactions are readily followed spectroscopically; and (*iv*) their co-ordination chemistry is expected to be 'simple'[12] without, for example, the complications due to tautomerism found with imidazoles.

Experimental

Materials.—All amines were purchased from Aldrich as hydrochloride salts except for ethanolamine which was purchased from Merck as the free amine. Solutions of the hydrochloride salts were standardised by potentiometric titration against $AgNO_3$. Ethanolamine was used as received.

† For convenience B_{12a} is abbreviated dmbzim–Co–H_2O, showing only the axial ligands and neglecting the overall charge.

Table 1 Acid dissociation constants for primary amines determined by potentiometric titration (I = 1.000 mol dm^{-3}). Standard errors are given in parentheses

	pK_a					
L	5	15	25	35 °C	ΔH/kJ mol^{-1}	ΔS/J K^{-1} mol^{-1}
NH_2OMe	5.190(6)	4.985(4)	4.745(1)	4.545(3)	35.3(1)	27.4(2)
NH_2OH	6.638(8)	6.392(6)	6.143(3)	5.911(8)	39.9(4)	16(1)
$NH_2CH_2CO_2Me$	8.213(4)	7.901(7)	7.621(2)	7.384(6)	45.5(6)	6(2)
NH_3	10.165(8)	9.820(5)	9.484(3)	9.178(3)	54.1(2)	0.0(7)
$NH_2CH_2CH(OH)CH_2OH$	10.141(6)	9.833(3)	9.509(3)	9.232(4)	50.1(6)	−14(2)
$NH_2(CH_2)_2OH$	10.414(4)	10.208(4)	9.785(1)	9.493(6)	49.4(1.8)	−21(6)
$NH_2(CH_2)_3OH$	11.160(4)	10.842(10)	10.435(6)	10.115(8)	58.1(1.8)	−5(4)
NH_2Me	11.671(9)	11.294(3)	10.941(1)	10.618(3)	57.7(1)	−16.1(5)
NH_2Pr	11.650(12)	11.286(8)	10.930(9)	10.603(5)	57.4(3)	−17(1)

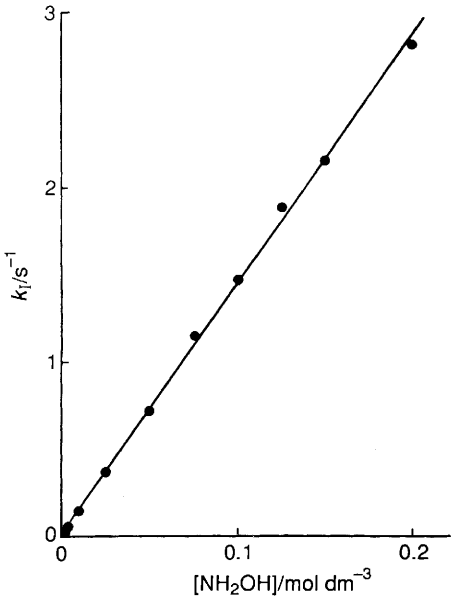


Fig. 1 Plot of the pseudo-first-order rate constant, k_I, as a function of [NH_2OH] at pH 6.77 and 25.0 °C. The gradient, k_{II}^{obs} = 14.3 ± 0.3 dm^3 mol^{-1} s^{-1}

Hydroxocobalamin (routinely stored at −20 °C) was purchased from Rousell and shown by HPLC to be >99% pure. All reactions were performed at a constant ionic strength of 1.00 mol dm^{-3} maintained with KCl (Merck). Water was twice distilled in an all-glass distillation unit and further purified by passage through a Millipore MilliQ system (18 MΩ cm). Buffer reagents [tris(hydroxymethyl)methylamine–HCl, used to buffer at around pH 9; phosphate for around pH 7] were purchased either from BDH or from Merck. Sodium hydroxide solutions (Merck, Titrisol®) were standardised against potassium hydrogenphthalate, and HCl (Merck, Titrisol®) was standardised against this solution.

Equipment.—The pH of solutions was measured with a Metrohm 605 ion/pH meter and a Metrohm 6.0210.100 micro combination glass electrode calibrated against standard buffers, all maintained at the relevant temperature with a thermostatted circulating water-bath. For potentiometric titrations, a Metrohm model 6.0203.001 combination glass electrode was used. Spectra were recorded on a Cary 2300 UV–VIS spectrometer equipped with thermostatted cuvette holders. Kinetic experiments were recorded either on this spectrometer using 1.00 cm pathlength cuvettes or on a Hi-Tech Scientific SF-51 stopped-flow spectrometer system (cell pathlength 1.00 cm) interfaced through a DAS-50 A/D board with an IBM-type PC, as appropriate, depending on the speed of the reaction. In both cases temperature was maintained constant by a circulating water-bath.

Methods.—The pK_a values of the ligands studied were determined at four temperatures (5, 15, 25 and 35 °C) by potentiometric titration of 25.00 cm^3 of *ca.* 50 mmol dm^{-3} solutions (in a solution of total ionic strength, I = 1.000 mol dm^{-3}, maintained with KCl) with standardised (*ca.* 1 mol dm^{-3}) NaOH or HCl, as appropriate, in a titration cell essentially as described by Martell and Motekaitis.[13] The electrode response (mV), as a function of titrant concentration, was analysed using the computer program EQUILIBRIA[14] from which the acid dissociation constants were obtained.

Reaction rates were studied by observing for at least five half-lives the increase in absorbance at 358 nm on formation of the amino complex under pseudo first-order conditions ([B_{12a}] ≈ 80 μmol dm^{-3}, ligand concentration as given in Table 2, I = 1.00 mol dm^{-3}, KCl). Pseudo-first-order rate constants, k_I, were obtained by curve-fitting the absorbance–time traces using a non-linear least-squares program employing a Newton–Raphson procedure. Second-order rate constants, k_{II}^{obs}, were obtained from a linear least-squares plot of k_I *versus* [L]. The intercepts of such plots were always within two standard deviations of the origin which means that k_r [equation (1)] is very small (<10^{-3} s^{-1}). The activation parameters $\Delta H^‡$ and $\Delta S^‡$ were determined from the slopes and intercepts, respectively, of plots of ln ($k_{II}h/k_BT$) against T^{-1}, where k_{II} is defined below [equation (3)] and h and k_B are the Planck and Boltzmann constants, respectively.

Results

The acid dissociation constants of the amines [equation (2)],

$$\underset{\mathbf{I}}{R\text{–}NH_3^+} \overset{K_a}{\rightleftharpoons} \underset{\mathbf{II}}{R\text{–}NH_2} + H^+ \qquad (2)$$

and ΔH and ΔS determined from their variation with temperature, are listed in Table 1.

The reactions generally gave simple monophasic kinetics and plots of k_I against ligand concentration were linear with intercepts not significantly different from zero. An example of such a plot for hydroxylamine at 25 °C is shown in Fig. 1.

At high temperature (50 °C) hydroxylamine causes reduction of Co^{III} to Co^{II}. However, the reduction reaction is at least an order of magnitude slower than co-ordination and manifests itself as a downward drift in A_∞, the equilibrium absorbance after equilibrium has been reached, as the γ band of dmbzim–Co–NH_2OH shifts on reduction. Pseudo-first-order rate constants were therefore obtained by fitting the absorbance–time trace to equation (3). A double exponential function gave

$$A = A_0 \exp(-k_I t) + mt + A_\infty \qquad (3)$$

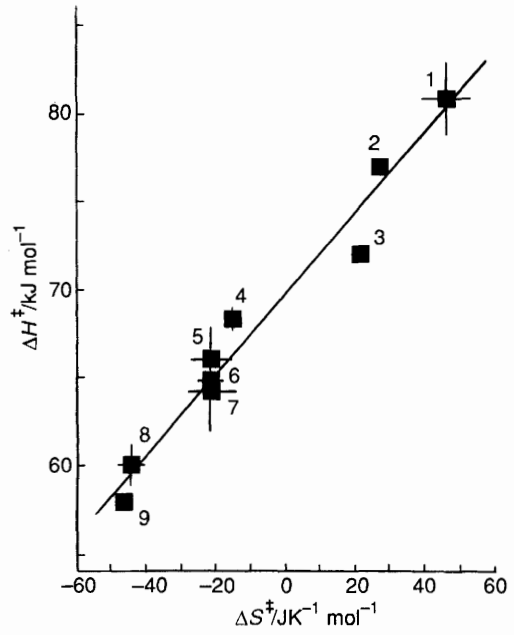


Fig. 2 Relationship between $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ for the reaction of aquocobalamin with neutral primary amines: 1 NH_2OMe, 2 NH_3, 3 NH_2OH, 4 $NH_2(CH_2)_3OH$, 5 $NH_2CH_2CO_2Me$, 6 NH_2Pr, 7 NH_2Me, 8 $NH_2CH_2CH(OH)CH_2OH$ and 9 $NH_2(CH_2)_2OH$

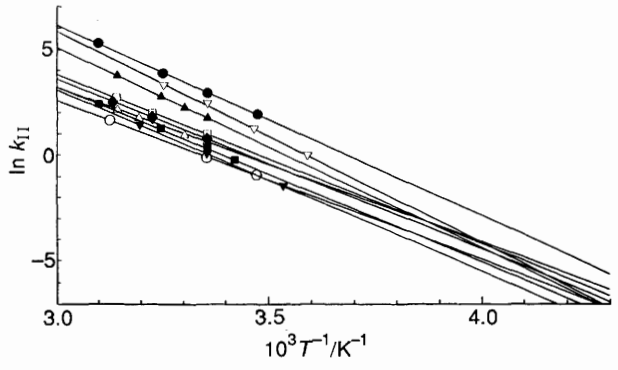


Fig. 3 Plot of ln k_{II} against $10^3\ T^{-1}$ for (●) NH_2OH, (▽) NH_2OMe, (▲) NH_3, (□) NH_2Me, (◆) NH_2Pr, (△) $NH_2(CH_2)_2OH$, (■) $NH_2CH_2CO_2Me$, (▼) $NH_2(CH_2)_3OH$ and (○) $NH_2CH_2CH(OH)CH_2OH$. The failure of the extrapolated straight lines to meet at a single point indicates the absence of an isokinetic relationship for this series of ligands

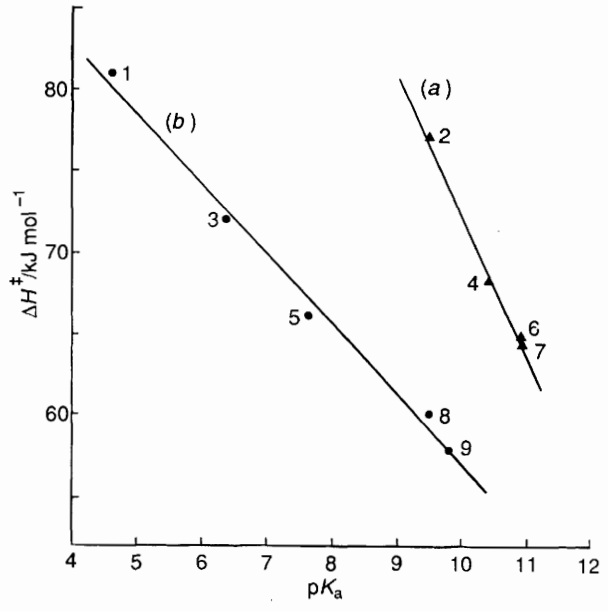


Fig. 4 Relationship between $\Delta H^{\ddagger}$ for the reaction of aquocobalamin with a primary amine, L, and the pK_a of L. Numbers as in Fig. 2

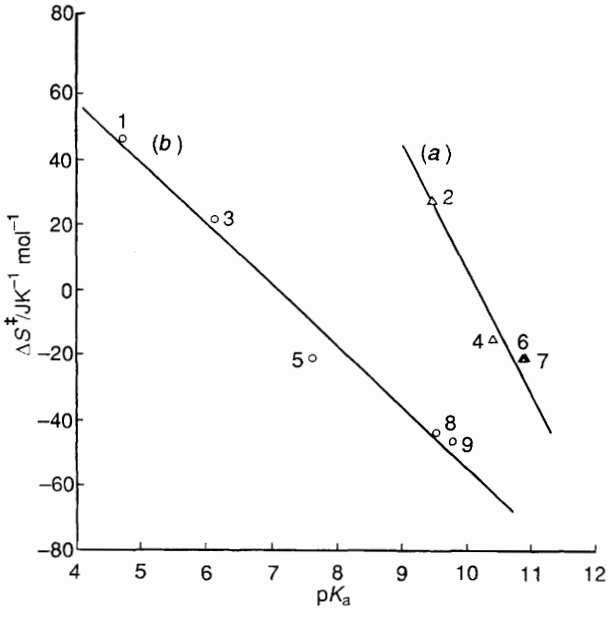


Fig. 5 Relationship between $\Delta S^{\ddagger}$ for the reaction of aquocobalamin with a primary amine, L, and the pK_a of L. Numbers as in Fig. 2

unreliable results because of the slowness of the second reaction.

The kinetics of ligand addition to B_{12a} are strongly dependent upon pH since (*i*) only the deprotonated amine **II** [equation (2)] will co-ordinate to Co^{III}, and (*ii*) hydroxocobalamin is inert to substitution.[1,8,15] Values of k_{II}^{obs} obtained from such plots were therefore modified [equation (4)] to take into account

$$k_{II} = k_{II}^{obs}(1 + [H^+]/K_I)(1 + K_{Co}/[H^+]) \qquad (4)$$

both the ionisation of Co^{III}-bound H_2O (pK_{Co} = 8.10 at 25 °C, I = 1 mol dm^{-3}, ΔH = 28.6 kJ mol^{-1}, ΔS = −59 J K^{-1} mol^{-1})[1] and the ionisation of the ligand, while also taking into account the temperature variation of these dissociation constants, to obtain a pH-independent second-order rate constant, k_{II}. The values of k_{II}^{obs}, k_{II}, $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ obtained are listed in Table 2.

An examination of the activation parameters in Table 2 reveals compensating changes in $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$, *i.e.*, these quantities decrease monotonically (Fig. 2). For the range of ligands studied, $\Delta H^{\ddagger}$ values range from *ca.* 81 to 58 kJ mol^{-1} while $\Delta S^{\ddagger}$ values range from *ca.* +46 to −46 J K^{-1} mol^{-1}. A compensation effect is not necessarily statistically significant, however, since both quantities are derived from the same data set and are therefore not independent.[16,17] When ln k_{II} is plotted against T^{-1} (Fig. 3), it is clear that there is no general isokinetic relationship for the series of ligands studied, *i.e.*, the (extrapolated) straight lines do not intersect at a common point, the isokinetic temperature, T_{iso} (a < 5% variation in T_{iso} is generally required[18]).

Correlations were found between the pK_a of the ligand (a measure of the donor power of the N atom towards the proton, and hence, to a first approximation, a measure of its donor power towards a metal ion) and the activation parameters (Figs. 4 and 5), with both $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ decreasing with increasing ligand pK_a. The ligands appear to fall into two distinct classes with NH_3, $NH_2(CH_2)_3OH$, NH_2Me and NH_2Pr in the first class [Class (*a*)], and NH_2OMe, NH_2OH, $NH_2CH_2CO_2Me$, $NH_2CH_2CH(OH)CH_2OH$ and $NH_2(CH_2)_2OH$ in the second class [Class (*b*)].

Table 2 Rate constants as a function of temperature for reactions of aquocobalamin with primary amines L

L	[L]/ mol dm^{-3}	T/ °C	pH	k_{II}^{obs}/ dm^3 mol^{-1} s^{-1}	k_{II}/ dm^3 mol^{-1} s^{-1}	$\Delta H^{\ddagger}$/ kJ mol^{-1}	$\Delta S^{\ddagger}$/ J K^{-1} mol^{-1}	Experimental technique
NH_2Me	0.006–0.35	5.5	6.47	0.872 ± 0.021	0.927 ± 0.022			*a*
		15.5	6.44	3.16 ± 0.02	3.31 ± 0.02			
		25.0	7.03	10.2 ± 0.1	11.1 ± 0.1			
		34.4	6.38	24.9 ± 0.6	26.0 ± 0.6	80.9 ± 2.1	46 ± 7	
NH_2OH	0.0005–0.20	15.0	6.84	4.79 ± 0.07	6.75 ± 0.09			*b*
		25.0	6.77	14.3 ± 0.2	18.6 ± 0.2			
		34.9	6.75	39.7 ± 0.2	48.6 ± 0.3			
		50.0	6.73	164 ± 4	195 ± 5	72.0 ± 0.5	21 ± 2	
$NH_2CH_2CO_2Me$	0.025–0.25	19.7	7.80	0.272 ± 0.004	0.753 ± 0.011			*b*
		25.2	7.66	0.542 ± 0.008	1.44 ± 0.02			
		35.1	7.42	1.39 ± 0.01	3.47 ± 0.02			
		50.1	7.04	4.65 ± 0.17	11.0 ± 0.4	66.1 ± 1.8	−21 ± 6	
NH_2	0.04–0.50	25.0	8.75	0.163 ± 0.005	5.74 ± 0.18			*b*
		29.9	8.64	0.312 ± 0.004	9.58 ± 0.12			
		35.0	8.50	0.595 ± 0.007	16.0 ± 0.1			
		45.2	8.24	2.11 ± 0.03	44.1 ± 0.7	77.0 ± 0.4	27 ± 1	
$NH_2CH_2CH(OH)CH_2OH$	0.01–0.11	15.0	9.23	$8.07 \times 10^{-3} \pm 0.13 \times 10^{-3}$	0.398 ± 0.007			*c*
		25.0	9.05	$2.24 \times 10^{-2} \pm 0.04 \times 10^{-2}$	0.874 ± 0.015			
		47.0	8.65	$2.10 \times 10^{-1} \pm 0.06 \times 10^{-1}$	5.29 ± 0.15	60.1 ± 1.2	−44 ± 4	
$NH_2(CH_2)_2OH$	0.001–0.40	25.0	9.20	0.0284 ± 0.009	1.79 ± 0.06			*c*
		30.0	9.05	0.0473 ± 0.0016	2.62 ± 0.09			
		40.0	8.74	0.132 ± 0.008	5.71 ± 0.36			
		45.0	8.76	0.205 ± 0.005	8.26 ± 0.23	57.9 ± 0.3	−46 ± 1	
$NH_2(CH_2)_3OH$	0.05–0.80	10.0	9.82	$4.85 \times 10^{-4} \pm 0.27 \times 10^{-4}$	0.225 ± 0.012			*c*
		25.0	9.39	$4.14 \times 10^{-3} \pm 0.01 \times 10^{-3}$	1.06 ± 0.00(2)			
		40.0	8.97	$2.69 \times 10^{-2} \pm 0.13 \times 10^{-2}$	4.03 ± 0.19	68.3 ± 0.6	−15 ± 2	
NH_2Me	0.05–0.50	25.0	9.84	$3.38 \times 10^{-3} \pm 0.06 \times 10^{-3}$	2.67 ± 0.05			*c*
		37.0	9.39	$1.39 \times 10^{-2} \pm 0.03 \times 10^{-2}$	6.98 ± 0.15			
		45.3	8.97	$3.97 \times 10^{-2} \pm 0.10 \times 10^{-2}$	15.1 ± 0.4	64.3 ± 2.3	−21 ± 7	
NH_2Pr	0.05–0.50	25.0	9.55	$2.92 \times 10^{-3} \pm 0.02 \times 10^{-3}$	2.16 ± 0.01			*c*
		37.1	9.33	$1.24 \times 10^{-2} \pm 0.02 \times 10^{-2}$	5.96 ± 0.09			
		46.0	8.95	$3.64 \times 10^{-2} \pm 0.11 \times 10^{-2}$	13.0 ± 0.6	64.8 ± 1.2	−21 ± 4	

[a] Stopped-flow + conventional spectroscopy. [b] Stopped flow. [c] Conventional spectroscopy.

Discussion

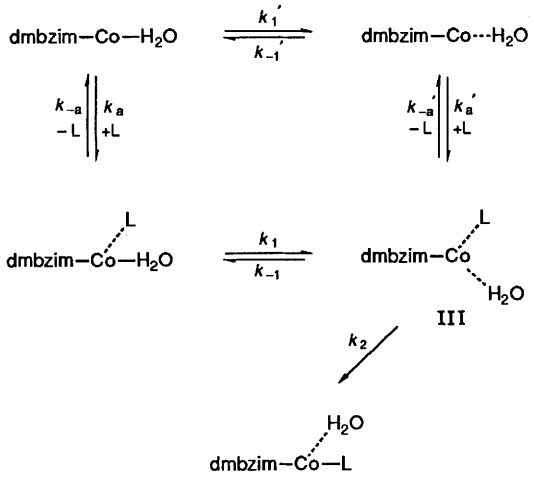
A number of reports (for example refs. 1, 3, 8, 9, 19–24) have appeared concerning the ligand substitution reactions of B_{12a}, but this appears to be the first comprehensive study of the kinetics of a closely related series of ligands with aquocobalamin. The present study was undertaken to determine whether correlations exist between the structure of the incoming ligand and the activation parameters for their reaction with aquocobalamin in order to help delineate factors which control these reactions. Previously available data, with the exception of our study of the reactions with small anionic and neutral ligands[3] and that of van Eldik and co-workers[9] dealing with azide and cyanoferrates, have been confined to a single temperature; there is no reason to suppose that comparisons of rate constants at a single temperature can offer any meaningful insight into the factors which control the rate of these reactions because of the likely existence of a compensation effect, as already demonstrated for CN^- and HCN.[1]

It was found that the reactions producing aminocobalamins [equation (1)] gave simple first-order kinetics, with the exception of NH_2OH at high temperature where reduction of Co^{III} to Co^{II} occurs; even in this case, the initial complexation reaction is much faster than reduction and rates of co-ordination could therefore be determined. In parallel experiments to determine the formation constants of aminocobalamins[10] it has been found that a single ligand L is co-ordinated by Co^{III}; it would appear, therefore, that the reactions studied are simple.

A compensation effect between $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ was found for the primary amines studied (Fig. 2). Although the linearity of such plots may be purely fortuitous, arising from random experimental error,[6,25] this is unlikely to be the case here since the errors are small compared to $\Delta(\Delta H^{\ddagger})$ and $\Delta(\Delta S^{\ddagger})$ observed. The existence of this compensation effect supports our belief that mechanistic conclusions drawn from comparisons of rate constants obtained at a single temperature should be treated with circumspection. It is interesting to note that, despite the apparently 'simple' nature of the reaction studied, there is no isokinetic relationship between members in the series (Fig. 3). Although there appears as yet to be no generally accepted explanation for the origin of isokinetic relationships,[17] their presence has been taken to indicate the operation of a single reaction mechanism,[6] and, conversely, deviations from such a relationship is indicative of a change in mechanism (for example).[26–28] This suggests that in the present case the reaction of B_{12a} with primary amines proceeds through multiple pathways.

Although the appearance of Fig. 2 might suggest that all nine ligands studied fall in the same series, when the activation parameters are correlated with the ligand pK_a (Figs. 4 and 5) it is clear that they fall into two distinct series. There may be different ways of interpreting these findings, the following of which is favoured. In their analysis of the mechanism of the ligand substitution reactions of octahedral metal complexes in general, and the cobalamins in particular, Reenstra and Jencks[8] contended that a purely D mechanism was unlikely to be operative in a co-ordinating solvent such as H_2O. Apparently all ligand substitution reactions of B_{12a} reported to date are second-order with rate-limiting addition of L. The substitution of H_2O proceeds through the (unstable) five-co-ordinate intermediate **III** [equation (5)]; for a D mechanism [upper pathway, Scheme 1] to be operative, therefore, requires that H_2O adds to **III** fast compared to addition of L by the k_2 route, but slow compared to diffusion of L away from **III** ($k_{-a} > k_{-1}$); consequently, an I_d mechanism is the most likely route.

The activation parameters for these reactions will be



Scheme 1 Reaction of aquocobalamin with incoming ligand, L

critically affected by any nucleophilic participation, however small, of L in the transition state. The ability of the incoming ligand to participate in the transition state is expected to increase with its donor power; this should be manifest as a decrease in $\Delta H^{\ddagger}$ (as Co–L bond formation compensates for Co–O bond breaking) and a parallel decrease in $\Delta S^{\ddagger}$ (due to loss of freedom of motion of the ligand). This is an explanation for the compensation effect observed in Figs. 4 and 5, *viz.*, as the pK_a of L increases, both $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ decrease.

Also of interest is (*i*) the absence of an isokinetic relationship for the ligands and (*ii*) the apparent existence of two distinct classes of ligands where, for a given pK_a value, a reaction with a ligand in Class (*b*) has a smaller $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ than one with a ligand in Class (*a*). One of the notable features of the architecture of the corrin ring is the presence of amide side-chains on its periphery directed towards the upper face of the ring, and hence towards the route which an incoming ligand must follow to displace H_2O from the co-ordination sphere of the metal ion. Differences in interaction between these side-chains and various incoming ligands will lead to an apparently simple reaction (the substitution of H_2O by L) following multiple paths, and this would explain the absence of an isokinetic relationship.

Nevertheless, the ligands studied appear to fall into two distinct classes. A characteristic distinguishing ligands in Class (*b*) from those in Class (*a*) is the presence of a functional group, not more than two carbon atoms away from the co-ordinating amino functionality, capable of participating in hydrogen bonding. (Thus 3-aminopropane-1,2-diol falls into Class (*b*), but 3-aminopropan-1-ol falls into Class (*a*); the other Class (*a*) ligands do not contain groups capable of hydrogen bonding.) It is suggested that this feature is responsible for the differences in behaviour of the two ligands. The ability of a ligand to participate in hydrogen bonding with the amide side-chains is envisaged to result in an increase in its local concentration and hence increase its ability to compete with the solvent for the metal ion. Examination of molecular models suggests that if the hydrogen bonding functionality is greater than two carbon atoms from the amino group, interaction with the acetamide side-chains will not help position the amino functionality for interaction with the metal ion; hydrogen-bonding interactions of this type will therefore be unproductive. It is hoped to establish this conclusion more clearly using molecular mechanics techniques by adapting for the cobalamins the force field recently developed for iron porphyrins.[29] A study of the kinetics of the reaction of B_{12a} with NH_2OH, NH_2Me and NH_2NH_2 at 25 °C, $I = 0.20$ mol dm^{-3} has been reported previously.[11] Rate constants for the first two (21.5 and 1.05 dm^3 mol^{-1} s^{-1}, respectively) are in good agreement with the results of this study; a value of 6.1×10^2 dm^3 mol^{-1} s^{-1} was reported for NH_2NH_2. The differences found were also attributed to differential hydrogen bonding between the acetamide side-chains of the corrin ring and the incoming ligand.

The acetamide side-chains are therefore conceived as structural entities instrumental in discriminating between incoming ligands. This effect is not expected, for example, in porphyrins which lack substituents on the periphery of the macrocycle and experiments to examine this presumed lack of effects are being planned. Irrespective of whether a hydrogen-bonding interaction occurs or not, for a given class of ligands the greater the donor power of the amino group the greater the extent of its participation in the transition state, leading to the observed decrease in $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ with increasing pK_a.

This study has shown (*i*) that there is a linear relationship between entropies and enthalpies of activation for the reaction of neutral primary amines with B_{12a} but that the ligands studied do not show an isokinetic relationship; (*ii*) that $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ both decrease as the donor power of the amine increases; and (*iii*) that the amines studied fall into two distinct reactivity classes. While (*i*) and (*ii*) are rationalised on the basis of nucleophilic participation of the incoming ligand, L, in the transition state, (*iii*) is explained by hydrogen-bonding interaction of functional groups on the ligand with the acetamide side-chains of the corrin ring which are directed towards the route which an incoming ligand must follow.

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References

1 H. M. Marques, K. L. Brown and D. W. Jacobsen, *J. Biol. Chem.*, 1988, **266**, 12378.
2 H. M. Marques, T. J. Egan, J. H. Marsh, J. R. Mellor and O. Q. Munro, *Inorg. Chim. Acta*, 1989, **166**, 249.
3 H. M. Marques, *J. Chem. Soc., Dalton Trans.*, 1991, 339.
4 J. M. Pratt, *The Inorganic Chemistry of Vitamin B_{12}*, Academic Press, New York, 1972.
5 C. K. Poon, *Coord. Chem. Rev.*, 1973, **10**, 1.
6 J. F. Bunnett, in *Techniques of Chemistry. Vol. VI, Investigation of Rates and Mechanisms of Reactions, Part 1*, ed. C. F. Bernasconi, Wiley, New York, 1986, pp. 251–372.
7 N. R. de Tacconi, D. Lexa and J. M. Savéant, *J. Am. Chem. Soc.*, 1979, **101**, 467.
8 W. W. Reenstra and W. P. Jencks, *J. Am. Chem. Soc.*, 1979, **101**, 5780.
9 G. Stochel, R. van Eldik, H. Kunkely and A. Vogler, *Inorg. Chem.*, 1989, **28**, 4314.
10 H. M. Marques, unpublished work.
11 J. G. Toerien, M.Sc. Dissertation, University of the Witwatersrand, Johannesburg, 1986; J. M. Pratt and J. G. Toerien, unpublished work.
12 D. A. Baldwin, E. A. Betterton and J. M. Pratt, *J. Chem. Soc., Dalton Trans.*, 1983, 2217.
13 A. E. Martell and R. J. Motekaitis, *Determination and the Use of Stability Constants*, VCH, Weinheim, 1988.
14 P. W. Wade and R. D. Hancock, University of the Witwatersrand, Johannesburg, 1986.
15 J. B. Conn and T. G. Wartman, *Science*, 1952, **115**, 72.
16 O. Exner, *Nature (London)*, 1964, **201**, 488.
17 W. Linert and R. F. Jameson, *Chem. Soc. Rev.*, 1989, **18**, 477.
18 W. Linert, *Chem. Phys.*, 1989, **129**, 381.
19 W. C. Randall and R. A. Alberty, *Biochemistry*, 1967, **6**, 1520.
20 S. Balt, M. G. de Bolster, C. J. van Garderen, A. M. van Herk, K. R. Lammers and E. G. van der Velde, *Inorg. Chim. Acta*, 1985, **106**, 43.
21 D. A. Baldwin, E. A. Betterton and J. M. Pratt, *S. Afr. J. Chem.*, 1982, **35**, 173.
22 D. Thusius, *J. Am. Chem. Soc.*, 1971, **93**, 2629.
23 K. Kano, F. Nome and J. H. Fendler, *J. Chem. Soc., Dalton Trans.*, 1978, 1226.
24 W. C. Randall and R. A. Alberty, *Biochemistry*, 1966, **5**, 3189.
25 J. E. Leffler, *Nature (London)*, 1965, **205**, 1101.

26 H. C. Brashears, D. W. Setser and Y. C. Yu, *J. Phys. Chem.*, 1980, **84**, 2495.
27 C. Repond, J. M. Mayer, H. van de Waterbeemd, B. Testa and W. Linert, *Int. J. Pharm.*, 1987, **38**, 47.
28 Y. Sueishi and N. Nishimura, *Bull. Chem. Soc. Jpn.*, 1983, **56**, 2598.
29 R. D. Hancock, J. S. Weaving and H. M. Marques, *J. Chem. Soc., Chem. Commun.*, 1989, 1176.

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