Ligand Substitution Reactions of Aquacobalamin: Further Evidence for a Dissociative Interchange Mechanism, and Factors controlling Reaction Rates†

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The kinetics of the substitution of H_2O in aquacobalamin (vitamin B_{12a}) by the anionic ligands I^-, $S_2O_3^{2-}$, NO_2^-, SCN^- and N_3^- have been determined as a function of ligand concentration and temperature by stopped-flow spectrophotometry at constant ionic strength (2.20 mol dm^{-3}) and pH (6–7). The observed pseudo-first-order rate constants saturate to a limiting value, k_{sat}, at high ligand concentrations which is consistent with the reactions proceeding through a dissociative activation pathway. The value of k_{sat} depends on the entering ligand and the activation parameters for k_{sat}, $\Delta H^\ddagger$ and $\Delta S^\ddagger$, are directly correlated [$\Delta H^\ddagger$ varies from 26 ± 1 (I^-) to 83 ± 4 (N_3^-) kJ mol^{-1} while $\Delta S^\ddagger$ varies from −92 ± 4 (I) to 94 ± 13 (N_3^-) J K^{-1} mol^{-1}]. This is inconsistent with a limiting dissociative (D) mechanism which requires k_{sat}, and hence $\Delta H^\ddagger$ and $\Delta S^\ddagger$, to be independent of the identity of the entering ligand. The mechanism is therefore best described as a dissociative interchange (I_d) mechanism. There is a direct correlation between $\Delta H^\ddagger$ (and hence $\Delta S^\ddagger$) and the total Mulliken population (as determined by semi-empirical molecular orbital methods) of the donor atom of the entering ligand, but no correlation between these activation parameters and the cone angle subtended by the ligand at the metal atom. This suggests that for anionic ligands, in contrast with neutral N-donor ligands, electronic rather than steric effects are primarily responsible for controlling reaction rates. It has been further concluded that for anionic ligands capable of bonding through two different donor atom types the reaction occurs primarily through the donor atom with the higher electron density.

The ligand substitution reactions of aquacobalamin (vitamin B_{12a}, abbreviated to dmbzim–Co–H_2O, where dmbzim = 5,6-dimethylbenzimidazole) (Fig. 1) in which axially-co-ordinated H_2O is replaced by an entering ligand, L, have continued to attract attention. It has recently been demonstrated that at sufficiently high concentrations of L (L = pyridine,[1,2] hydroxylamine, methyl glycinate, 4-methylpyridine, imidazole or histamine[2]) plots of the observed rate constant for this process against [L] approach a limiting value, k_{sat}. The reactions therefore proceed through a dissociative activation pathway but there has been some disagreement about whether the intimate mechanism is a limiting dissociative (D) or an interchange dissociative (I_d) mechanism.[1,3–6]

In a limiting dissociative mechanism k_{sat} corresponds to the rate constant, k_1, for unimolecular dissociation of water from Co^{III} [equations (1) and (2)] whereas in a dissociative

$$\text{dmbzim–Co–H}_2\text{O} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{dmbzim–Co} + \text{H}_2\text{O} \quad (1)$$

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

interchange mechanism it corresponds to the interchange rate constant, k_4 [equations (3) and (4)].

$$\text{dmbzim–Co–H}_2\text{O} + \text{L} \overset{K}{\rightleftharpoons} \{\text{H}_2\text{O}\cdots\text{dmbzim–Co}\cdots\text{L}\} \quad (3)$$

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

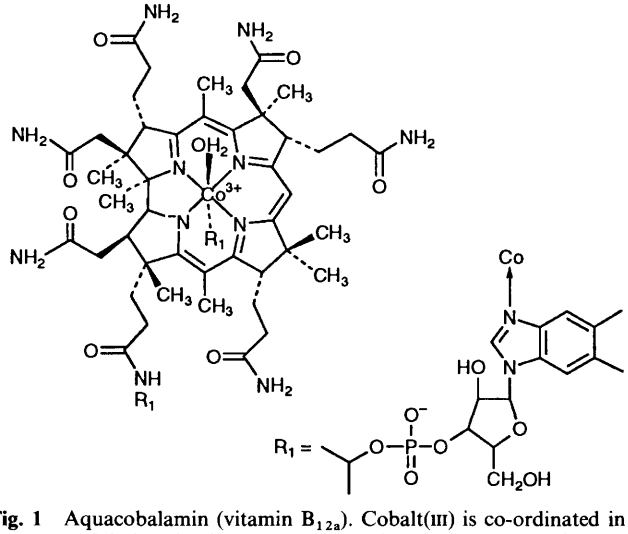

Fig. 1 Aquacobalamin (vitamin B_{12a}). Cobalt(III) is co-ordinated in the equatorial plane by the macrocycle corrin. The lower (α) and upper (β) faces of the molecule are defined by the respectively downward and upward projecting propionamide and acetamide side-chains. The α axial ligand is 5,6-dimethylbenzimidazole; the β ligand is H_2O. B_{12a} carries an overall charge of +1; there is a +3 charge of the metal, and −1 charges on a corrin N donor and the phosphate of the pendent nucleotide

A dependence of k_{sat} on L necessarily precludes a D mechanism; however, whether this dependence is observed or

† *Supplementary data available* (No. SUP 56980, 23 pp.): primary kinetic data. See Instructions for Authors, *J. Chem. Soc., Dalton Trans.*, 1994, Issue 1, pp. xxiii–xxviii.

not depends on the experimental conditions. In particular, unless sufficiently high concentrations of L are used, saturation will not be observed. It has been shown[2,5,7] that there is a direct correlation, and hence a compensation effect, between $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ for k_{sat} so that the values of k_{sat} for different ligands may coincidentally be very similar at a particular temperature. Since $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ were found to depend on incoming L (for example, $\Delta H^{\ddagger}$ varies from 101 to 23 kJ mol^{-1} while $\Delta S^{\ddagger}$ varies from 101 to -131 J K^{-1} mol^{-1} for histamine and hydroxylamine, respectively[2]), is was concluded[2] that the intimate mechanism must be an I_d mechanism and that there is participation by incoming L in the transition state.

For the neutral ligands NH_2OH, $NH_2CH_2CO_2Me$, pyridine, 4-methylpyridine and imidazole, good correlations were found between $\Delta H^{\ddagger}$ (and hence $\Delta S^{\ddagger}$, since $\Delta S^{\ddagger}$ and $\Delta H^{\ddagger}$ are correlated) and both the cone angle subtended by L at the metal ion at an arbitrary ligand-donor distance, and the molecular volume of L; there was no correlation between $\Delta H^{\ddagger}$ and the pK_a of L.[2] This suggested that steric, rather than electronic effects (insofar as these are measured by the pK_a of the ligand), were primarily responsible for controlling the extent of participation of L in the transition state. In an earlier report, where ligand concentrations used were relatively low and saturation was not observed, it was noted that the second-order rate constant, k_{II}, (which is a composite of K and k_4 if an I_d mechanism is followed by these ligands) for substitution of H_2O in B_{12a} by a series of imidazole derivatives appeared to depend on the pK_a of the incoming ligand, indicating that the reaction was controlled by the nucleophilicity of the entering ligand.[8] The activation parameters of k_{II} for a number of small anionic ligands are all very similar;[9] this led to the proposal that the extent of participation of ligands such as SCN^- and N_3^- in the transition state was minimal. More recently,[10] evidence has been presented that an I_d mechanism is operative in the binding of I^- by B_{12a} in methanol–water mixtures, in agreement with an earlier report.[11] No saturation effects were observed in aqueous solution, but relatively low concentrations of iodide (< 0.12 mol dm^{-3}) were used.[10]

In light of the accumulating evidence for saturation effects in the kinetics of the ligand substitution reactions of B_{12a}, and in order to provide further insight into factors which control the rate of these reactions, the kinetics of the substitution of H_2O in B_{12a} with I^-, SCN^-, $S_2O_3^{2-}$, NO_2^- and N_3^- have been re-examined and the results are reported in this paper.

Experimental

Hydroxocobalamin (> 99% pure, HPLC) was obtained from Roussel. Other reagents [KI, KCl, NaN_3 and $Na_2S_2O_3$ (Merck), $NaNO_2$ (BDH), NaSCN (Riedel de Haën)] were of the highest purity available and were used as received. Water was purified by distillation in an all-glass still and passage through a Millipore MilliQ system (18 MΩ cm). The pH of solutions was measured with a Metrohm model 6.0210.000 glass electrode and a Metrohm model 601 pH meter. Phosphate buffers were used to buffer the solutions at pH 6–7.

Preliminary spectroscopic work was carried out on a Cary 2300 or a Cary 1E spectrometer. Kinetic measurements were made on a Hi-Tech SF51 stopped-flow spectrophotometer interfaced through a DAS 50 A/D board to an IBM-type personal computer. The temperature (± 0.1 °C) was maintained by means of a circulating water bath.

Equilibrium constants for the binding of iodide to aquacobalamin were determined by mixing solutions of between 0.10 and 1.00 mol dm^{-3} in iodide (on mixing) and 50 μmol dm^{-3} B_{12a} (on mixing) buffered at pH 6.00 (0.100 mol dm^{-3} phosphate), I = 2.20 mol dm^{-3} (KCl) using the stopped-flow spectrophotometer. The equilibrium absorbance value was taken to be the maximum value of the absorbance reached at the monitoring wavelength (379 nm) before the absorbance decreased (see below). The equilibrium constant, K_{eq}, was determined by fitting the absorbance/concentration data to equation 5 where f(Co–H_2O), the fraction of B_{12a}, is given by $1/(1 + K_{eq}[I^-])$ and f(Co–I), the fraction of iodocobalamin, by $K_{eq}[I^-]/(1 + K_{eq}[I^-])$, using A_o, A_∞ and K_{eq} as fit parameters.

$$A = A_o \cdot f(\text{Co–H}_2\text{O}) + A_\infty \cdot f(\text{Co–I}) \quad (5)$$

All reactions were studied under pseudo-first-order conditions with B_{12a} concentrations between 50 and 100 μmol dm^{-3} and ligand concentrations (10–12 solutions) between 0.02 and 1.0 mol dm^{-3}. In the reactions with all ligands except $S_2O_3^{2-}$, the ionic strengths of the ligand and B_{12a} solutions were each maintained at 2.20 mol dm^{-3} using KCl which allowed for solutions up to 2.00 mol dm^{-3} in anionic ligand (*i.e.*, up to 1.00 mol dm^{-3} on mixing in the stopped-flow spectrophotometer) to be used. In the studies with $S_2O_3^{2-}$, the B_{12a} solutions were buffered with 50 mmol dm^{-3} phosphate. Because of the high ionic strength contribution from $S_2O_3^{2-}$, in studies with this ligand the ionic strengths of the reacting solutions could not be kept the same. Their ionic strengths were kept as close as possible, but ensuring that on mixing the total ionic strength was always 2.20 mol dm^{-3}. In this way, reactions of up to 0.70 mol dm^{-3} in $S_2O_3^{2-}$ could be studied. (Our use of chloride as ionic-strength adjustor has been questioned since it may compete for Co^{III}. We have examined the effect on the spectrum of 50 μmol dm^{-3} B_{12a} in neutral solution on addition of NaCl up to 4 mol dm^{-3}. Small spectral changes are indeed observed at $[Cl^-] \geq 2$ mol dm^{-3} with a gradual shift in the γ-band from 351 nm to 353 nm. We *estimate* that K_{eq} (for binding of Cl^-) ≤ 0.05 which means that at the highest concentration of Cl^-, *i.e.*, 2.00 mol dm^{-3}, used in the present work the cobalamin is ≤ 10% present as the chloro complex. Since this is of the order of the error in the kinetics results, this is not considered to be significant.)

The experimentally determined rate constants were determined by fitting a simple exponential function to the absorbance–time trace by means of a non-linear least-squares technique using a Newton–Raphson procedure and Marquardt's algorithm. The average error in k_4 varied from 5% (for N_3^-) to 11% (for SCN^-), and from 6% (I^-) to 23% (SCN^-) in K; the average error in k_{-4} (for I^-) was 9%.

The cone angle (the largest angle subtended at the metal ion by a co-ordinated ligand as measured from the extremes of the van der Waals radii of the atoms of the ligand) at an arbitrary ligand–metal distance of 2.2 Å was determined by molecular modelling using ALCHEMY III.[12] The van der Waals radii were obtained from Bondi;[13] an ionic radius of 2.16 Å was used for I^-.[14] The N–N bond length used for N_3^- was the mean of the azides of Na^+, Li^+ and Sr^{2+},[15] and the Co–N–N angle in co-ordinated N_3^- was assumed to be 126°, as seen in [FeL(N_3)(py)](H_2L = 5,10,15,20-tetraphenylporphyrin, py = pyridine).[16] The bond lengths in SCN^- were obtained from the reported crystal structure of NaSCN.[17] Values of 177 and 136° were used for the S–C–N and the Co–S–C (or Co–N–C) bond angles, respectively, as observed in [FeL(NCS)(py)].[18] The geometry of NO_2^- was that reported for $NaNO_2$,[19] and a Co–O–N bond angle of 102° (the value of the H–O–N angle in HNO_2)[20] was assumed. In the case of N-bound NO_2^-, a Co–N–O bond angle of 120° was employed {as seen[21] in [FeL'(NO_2)$_2$]$^-$} {H_2L' = 5,10,15,20-tetrakis-[2-(2,2-dimethylpropanamido)phenyl]porphyrin}. The geometry of $S_2O_3^{2-}$ was obtained from von Benda and von Benda[22] and Co–O–S and Co–S–S angles were taken as 135° {the Fe–O–S bond angle in [FeL(OSO_3H)]$^-$[23]}. The observed structures of pyridine,[24] imidazole[25] and NH_2OH[26] were used with Co occupying the position of the N lone pair in the molecular model; the cone angle of 4-methylpyridine was assumed to be identical to that of pyridine. In its sterically least demanding conformation with the side chain rotated away from the metal ion, the histaminium cation (where the imidazole N is deprotonated and the side chain amino group is protonated)

Table 1 Equilibrium constants for the binding of I^- to B_{12a} (I = 2.20 mol dm^{-3})

T/°C	log K_{eq}/dm^3 mol^{-1}
5.0	2.11 ± 0.04
10.0	1.94 ± 0.04
15.0	1.78 ± 0.05
25.0	1.46 ± 0.05

subtends the same cone angle as imidazole. The structure of methyl glycinate was determined using ALCHEMY III and the TRIPOS force field.[12]

The total Mulliken populations on the ligand donor atoms were obtained by semi-empirical MO calculations, performed with the PM3 model,[27,28] a reparameterisation of the MNDO (modified neglect of diatomic overlap) model developed by Dewar and co-workers,[29–31] using MOPAC,[32] available as part of the SYBYL program package.[12]

Results

The equilibrium constants for binding of I^- to B_{12a} as a function of temperature are given in Table 1. From a plot of ln K_{eq} against T^{-1} (not shown), it was found that $\Delta H = -51.3 \pm 0.4$ kJ mol^{-1} and $\Delta S = -144 \pm 3$ J K^{-1}. The value of K_{eq} at 25 °C is in good agreement with that previously reported (K_{eq} = 32 dm^3 mol^{-1}).[33] Since hydroxocobalamin is known to be kinetically inert,[3,6,7,34] the observed rate constants were converted to pH-independent rate constants by multiplying by a factor $(1 + K_{Co}/[H^+])$ where pK_{Co} is the temperature-dependent acid dissociation constant of co-ordinated H_2O in B_{12a} (ΔH = 28.6 ± 0.3 kJ mol^{-1} and $\Delta S = -59.0 \pm 1.2$ J K^{-1} mol^{-1}).[34]

The reactions with $S_2O_3^{2-}$, NO_2^-, SCN^- and N_3^- go essentially to completion under the conditions employed, since the equilibrium constants (log K_{eq} = 3.9, 5.4, 3.1 and 4.9, respectively)[35] are relatively large. In the case of I^-, however, log K_{eq} is small (Table 1); the observed rate constant will be the sum of the forward and reverse rate constants. The values of k_{obs} were therefore corrected for the reverse rate constant by multiplying by a factor $K_{eq}/(1 + K_{eq})$ where K_{eq} is the temperature-dependent equilibrium constant for formation of iodocobalamin.

In the case of NO_2^- and N_3^- simple exponential kinetics were observed. With I^-, the initial rapid change in absorbance accompanying co-ordination of I^- is followed by a comparatively slow ($t_{\frac{1}{2}}$ of the order of minutes) further change in absorbance due to oxidation of I^- catalysed by B_{12a}.[36] In the reactions with $S_2O_3^{2-}$ and SCN^- the kinetics were distinctly biphasic. The subsequent, slower reactions accounted for less than 15% of the total signal change observed, and were independent of ligand concentration (observed rate constant = 0.15 ± 0.04 s^{-1} at 25 °C for six solutions of $S_2O_3^{2-}$ with concentration between 0.20 and 0.70 mol dm^{-3}; observed rate constant = 4.5 ± 0.1 s^{-1} at 25 °C for 6 solutions of SCN^- with concentration between 0.30 mol dm^{-3} and 0.90 mol dm^{-3}). Since the slow reactions were, at the lowest concentration of ligand studied, 13 and 4.5 times slower than the fast reaction with $S_2O_3^{2-}$ and SCN^-, respectively, the latter could be studied without significant interference from the former. The probable origin of these slower reactions is discussed below.

The rate constants determined as a function of ligand concentration and temperature are available as supplementary material (SUP 56980). The values of the pH-corrected rate constants, k_{obs}, are plotted against ligand concentration [L] in Fig. 2; small, but significant, curvature is seen.

As previously discussed,[2] in aqueous solutions equations (6) and (7) pertain for a D and an I_d mechanism, respectively. The

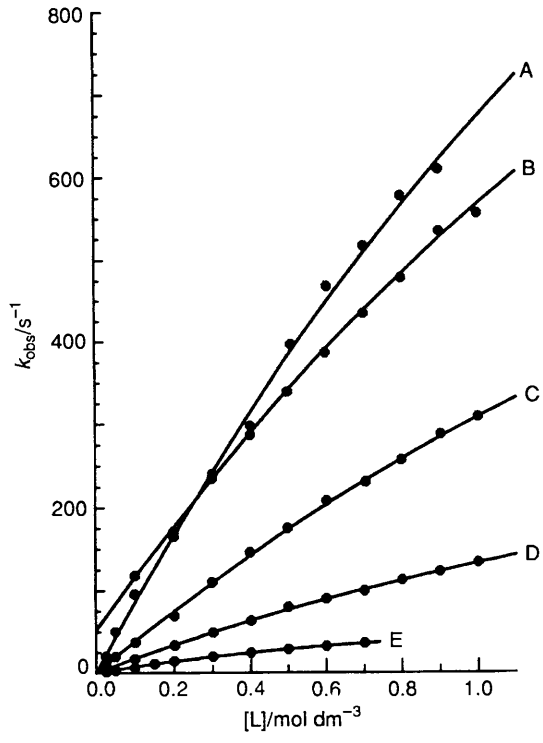

Fig. 2 Plot of the observed rate constant, k_{obs}, as a function of ligand concentration for the reaction of B_{12a} (25 °C, I = 2.20 mol dm^{-3}) with SCN^- (A), I^- (B), N_3^- (C), NO_2^- (D) and $S_2O_3^{2-}$ (E). The solid lines are least squares fits of equation (7) to the experimental data

$$k_{obs} = \frac{k_1k_2[L] + k_{-1}k_{-2}}{k_{-1} + k_2[L]} \quad (6)$$

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

two equations can be written in exactly the same form [*viz.* $k_{obs} = (a[L] + b)/(1 + c[L])$, with limiting rate constant a/c where $a = (k_1k_2/k_{-1})$ or $(k_4 + k_{-4})K$ and $c = k_2/k_{-1}$ or K, for the D and I_d mechanisms, respectively; $b = k_{-2}$ or $k_{-4} = 0$ if the reverse rate constants are insignificant]. The experimental data were fitted with this equation as objective functions and a,b and c as parameters. Data points were weighted as $1/\sigma^2$ where σ is the standard deviation of between 5 and 8 measurements of the rate constant, expressed as a percentage of the mean observed rate constant. The fitted results are given in Table 2. The limiting rate constant is clearly dependent on the identity of L [for example, at 25 °C the values (in s^{-1}) are 2800 ± 200 (SCN^-); 2200 ± 120 (I^-); 1370 ± 70 (N_3^-); 520 ± 20 (NO_2^-); 100 ± 10 ($S_2O_3^{2-}$)]; an I_d mechanism is therefore indicated and the appropriate equation relating the observed rate constant to [L] is equation (7).

Because the extent of curvature is in all cases very small, the determination of k_{sat} requires extrapolation well above accessible ligand concentrations; this brings into question the reliability of the fits of equation (7) to the data. Equation (7) was re-written as the linear equation (8) and the results obtained

$$\frac{1}{k_{obs}} = \frac{1}{k_4K[L]} + \frac{1}{k_4} \quad (8)$$

from linear least squares fit of the data to this equation are also listed in Table 2. One of the problems associated with reciprocal plots is the excessive weighting which the data points corresponding to low ligand concentrations will carry. Even small errors in these values will lead to significant skewing of

Table 2 Rate and equilibrium constants for the co-ordination of the ligands by aquacobalamin *

		Determined from equation (7)			Determined from equation (8)		Average	
Ligand	T/° C	k_4/s^{-1}	k_{-4}/s^{-1}	K/dm^3 mol^{-1}	k_4/s^{-1}	K/dm^3 mol^{-1}	k_4/s^{-1}	K/dm^3 mol^{-1}
N_3^-	5.0	115	—	0.62	120	0.59	120	0.61
	10.0	207	—	0.40	204	0.41	206	0.41
	14.9	355	—	0.50	340	0.53	350	0.52
	20.0	730	—	0.33	750	0.32	740	0.33
	24.7	1370	—	0.29	1310	0.31	1340	0.30
SCN^-	4.9	390	—	0.32	380	0.34	380	0.33
	10.0	570	—	0.42	570	0.43	570	0.43
	14.9	940	—	0.36	940	0.37	940	0.37
	20.0	1720	—	0.29	1510	0.37	1620	0.33
	24.9	2850	—	0.31	2940	0.30	2900	0.31
$S_2O_3^{2-}$	15.1	51	—	0.54	40	0.8	50	0.7
	25.1	100	—	0.8	90	0.91	90	0.9
	34.3	200	—	0.8	190	0.9	190	0.9
	45.2	390	—	0.89	390	0.87	390	0.9
NO_2^-	5.1	78	—	0.31	75	0.33	77	0.32
	15.1	220	—	0.31	210	0.31	210	0.31
	25.1	520	—	0.37	500	0.33	510	0.35
	35.2	990	—	0.49	960	0.51	980	0.50
I^-	5.1	1060	7.7	0.08	1030	0.09	1050	0.09
	10.0	1250	13.7	0.20	1210	0.21	1230	0.21
	15.0	1510	23.2	0.16	1500	0.16	1500	0.16
	25.0	2000	81.7	0.20	1990	0.34	2000	0.27

* Errors are standard errors of estimate.

Table 3 Activation parameters for the reaction of the ligands with aquacobalamin [a]

	k_4/s^{-1}		k_{-4}/s^{-1}		
Ligand	$\Delta H^{\ddagger}$/kJ mol^{-1}	$\Delta S^{\ddagger}$/J K mol^{-1}	$\Delta H^{\ddagger}$/kJ mol^{-1}	$\Delta S^{\ddagger}$/J K mol^{-1}	R^{2} [b]
N_3^-	83(4)	94(13)			0.9940
SCN^-	68(4)	48(13)			0.9914
NO_2^-	58(3)	0(12)			0.9926
$S_2O_3^{2-}$	49(2)	−42(6)			0.9977
I^-	26(1)	−92(4)			0.9959
			79(3)	58(11)	0.9971
Histaminium [c,d]	101(4)	101(14)			
NH_2OH [d]	23(4)	−131(12)			
Methyl glycinate [d]	60(3)	−43(10)			
Pyridine [d]	80(2)	41(1)			
4-Methylpyridine [d]	78(3)	32(9)			
Imidazole [d]	70(4)	19(12)			

[a] Errors (in parenthesis) are standard errors of estimate. [b] R^2 of plot of $\ln(k_4h/k_BT)$ against T^{-1}. [c] Histamine with protonated pendent amino group. [d] Ref. 2.

Table 4 Calculated [a] and crystallographically observed geometry of the ligands

Ligand	Parameter [b]	Calculated	Observed	Ref.
SCN^-	S–C	1.607	1.678 [c]	17
	N–C	1.179	1.200	
	S–C–N	180	179	
N_3^-	N–N	1.168	1.176 [d]	15
	N–N–N	180	180	
NO_2^-	N–O	1.234	1.240 [e]	19
	O–N–O	115.7	114.9	
$S_2O_3^{2-}$	S–S	2.121	1.994 [f]	22
	S–O	1.555	1.471	19
	O–S–O	109	109	19
	S–S–O	111	110	19

[a] By the PM3 model. [b] Bond distances in Å, angles in degrees. [c] In NaSCN. [d] Mean of azides of Na^+, Li^+ and Sr^{2+}. [e] In $NaNO_2$. [f] In $Na_2S_2O_3$.

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 concentrations) were used in fitting equation (8). As Table 2 shows, there is satisfactory agreement in the values of k_4 and K obtained by the two methods and their average was used in plots (not shown) of $\ln(k_4h/k_BT)$ against T^{-1}, where h and k_B are the Planck and Boltzmann constants, respectively, to determine $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$. The values obtained are listed in Table 3. The values of these activation parameters previously obtained [2] for other ligands are included in the Table.

The geometry of the anions calculated using the PM3 model is generally in good agreement with that observed crystallographically (Table 4). The total Mulliken populations [37] (*i.e.* the total valence electron density) on the donor atoms of the eleven ligands for which saturation kinetics in their reaction with B_{12a} are either reported here or have been previously reported [2] are listed in Table 5. The cone angles (see above) subtended at Co^{III} in B_{12a} at an arbitrary Co–L bond length of 2.2 Å, taken to be a reasonable estimate of the metal–ligand distance in the transition state, are also listed in Table 5.

Discussion

An examination of Fig. 2 and the activation parameters listed in Table 3 clearly shows that the saturating rate constant, k_{sat},

Table 5 Total Mulliken populations of donor atoms as determined using the PM3 model, and cone angles subtended at Co^{III} at an arbitrary metal–ligand distance of 2.2 Å

Ligand	Donor atom	Total Mulliken population	Cone angle/°
N_3^-	N	5.945	102
SCN^-	S	6.464	90
	N	5.383	95
NO_2^-	N	4.679	97
	O	6.660	116
$S_2O_3^{2-}$	S	7.234	125
	O	6.947	158
I^-	I	8.000	89
Histaminium	N	5.089	123
NH_2OH	N	5.010	102
Methyl glycinate	N	5.095	130
Pyridine	N	5.093	131
4-Methylpyridine	N	5.105	131
Imidazole	N	5.131	123

depends on the incoming ligand, L. This kinetic discrimination by Co^{III} in B_{12a} is good evidence for the nucleophilic participation by L in the transition state, which means that the ligand substitution reactions of B_{12a} occur through a dissociative interchange mechanism; k_{sat} may therefore be equated with the elementary rate constant k_4 of equation (4), the rate constant for the interchange of L and H_2O on Co^{III}. It had previously been shown[9] that the activation parameters for the apparent second-order rate constant, k_{II} (a composite of k_4 and K) were similar which suggested that the extent of participation (if any) by these ligands in the transition state was very similar. The present results therefore highlight the problem inherent in attempting to make mechanistic deductions based on measurements on anything other than elementary rate constants.

There is as yet no satisfactory explanation for the factors which control the formation of the initial outer-sphere complex between B_{12a} and an entering ligand. As recently pointed out,[38] no obvious correlation exists between the magnitude of the equilibrium constant for formation of the outer-sphere complex [K in equation (3)] and properties of the entering nucleophile such as charge and nucleophilicity. The reaction between B_{12a} and neutral and charged ligands has the same rate law with similar values for K [at 25 °C, for neutral ligands,[2] K = 0.25 (hydroxylamine), 2.4 (pyridine), 1.7 (methyl glycinate), 3.3 (4-methylpyridine), 0.65 (imidazole), and 0.49 (histaminium); from this work, for ligands with a −1 charge, 0.29 (N_3^-), 0.31 (SCN^-), 0.37 (NO_2^-) and 0.27 (I^-); and for the 2− ligand $S_2O_3^{2-}$, 0.81 $dm^3\ mol^{-1}$]. The interaction between the entering ligand and B_{12a} may depend on a number of factors, including electrostatic attraction between an anionic ligand and cationic B_{12a},[38] hydrogen bonding with the acetamide side-chains,[9] and hydrophobic interactions between the corrin ring and π systems of ligands such as pyridine. Clearly more data are required (especially with cobalamins containing modified side-chains) before any meaningful conclusions are possible.

A shown in Fig. 3, there is a good correlation between $\Delta H^\ddagger$ and $\Delta S^\ddagger$ for k_4 for the eleven ligands for which this rate constant has been measured. This compensation effect explains why the rate constants at a given temperature do not differ very greatly, an observation which has been used to justify assigning a limiting D mechanism to these reactions.[39] As we have previously pointed out,[2] this correlation accords with an I_d mechanism; it is envisaged that as the extent of participation by L in the transition state increases so $\Delta H^\ddagger$ decreases with bond making compensating for bond breaking, and consequently $\Delta S^\ddagger$ also decreases because of loss of degrees of freedom of the incoming ligand.

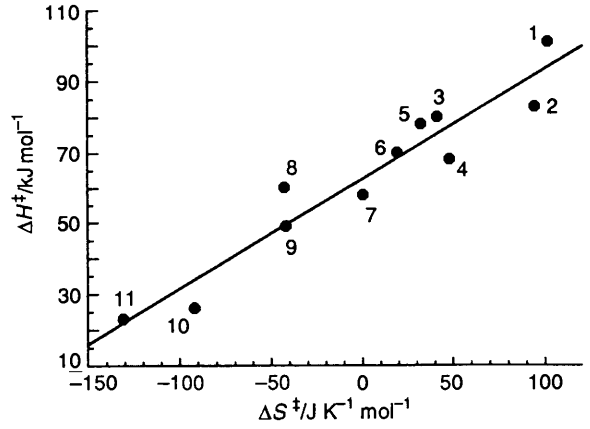

Fig. 3 The correlation between $\Delta H^\ddagger$ and $\Delta S^\ddagger$ (R^2 = 92.9%) of the saturating rate constant for substitution of H_2O in B_{12a} by histaminium (1); N_3^- (2); pyridine (3); SCN^- (4); 4-methylpyridine (5); imidazole (6); NO_2^- (7); methyl glycinate (8); $S_2O_3^{2-}$ (9); I^- (10) and NH_2OH (11)

Simple kinetics were observed with N_3^-, NO_2^- and I^- (other than the slow oxidation of I^- by B_{12a} as discussed above), but biphasic kinetics were seen with $S_2O_3^{2-}$ and SCN^-. Biphasic kinetics with SCN^- have been reported previously[40] and were attributed to formation of linkage isomers with bonding through both S and N; it was assumed that the S-bound product was the major component in solution. This is supported by a report by Pratt[35] (quoting a personal communication with Hodgkin) that X-ray crystallographic studies have shown SCN^- is bound through S. Because of the spectral similarities of the complexes, sulfur-containing ligands such as SO_3^{2-} and $S_2O_3^{2-}$ are generally accepted to co-ordinate through S[33] but studies of the binding of SO_3^{2-} to hydroxocobalamin at pH 14 were found to give rise to two products before slow conversion to S-bonded sulfitocobalamin.[41] We therefore attribute the observed biphasic kinetics in the reactions of B_{12a} with SCN^- and $S_2O_3^{2-}$ to the formation of a presumably minor component of N- and O-bound complexes of these ligands, respectively, which, in a slower step which is independent of ligand concentration, rearrange to the expected S-bound complexes. Whether NO_2^- is co-ordinated through N or O appears not to be known. The observation of monophasic kinetics with this ligand in this work suggests that either the thermodynamically stable product is the only one formed to any appreciable extent in solution (unless the N- and O-bound complexes coincidentally have very similar spectra) or, as suggested by a referee, that the rate of isomerisation for the putative NO_2^- isomers is very fast.

In addition to elucidating the mechanism of the ligand substitution reactions of B_{12a} we are interested in exploring the factors which control the rates of these reactions and have previously identified cases where either electronic (ligand nucleophilicity)[8] or steric (ligand size; cone angle subtended at the metal ion)[2] factors appear to be important. The present results provide a further opportunity of examining such possibilities.

In the discussion that follows, reference is made to correlations of k_4 with $\Delta H^\ddagger$; since $\Delta H^\ddagger$ and $\Delta S^\ddagger$ are themselves closely correlated (Fig. 3) it follows that similar correlations are found for $\Delta S^\ddagger$ as well. We have found no correlation between $\Delta H^\ddagger$ and the cone angle subtended by the five anionic ligands studied here (Fig. 4). The possibility of linkage isomerism is taken into account in the figure by presenting the cone angles for S- and N-bound SCN^-, N- and O-bound NO_2^-, and S- and O-bound $S_2O_3^{2-}$. The figure shows the correlation (R^2 = 0.958) between cone angle and the neutral N-donor ligands NH_2OH, methyl glycinate, pyridine, 4-methylpyridine and imidazole; histaminium was omitted from this correlation for reasons discussed below. Fig. 4 suggests, therefore, the steric

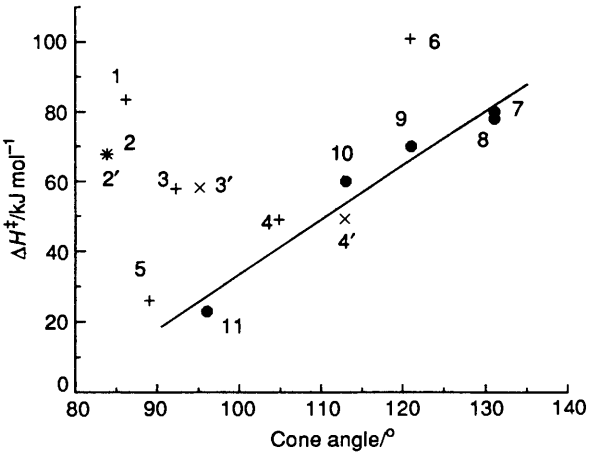

Fig. 4 Dependence of $\Delta H^{\ddagger}$ for k_4 on the cone angle subtended at the metal ion. N_3^- (1); S- and N-bound SCN (2 and 2′); O- and N-bound NO_2^- (3 and 3′); S- and O-bound $S_2O_3^{2-}$ (4 and 4′); I^- (5); histaminium (6); pyridine (7); 4-methylpyridine (8); imidazole (9); methyl glycinate (10); NH_2OH (11). The straight line is the least squares line through the data points for the neutral N-donor ligands (7–11)

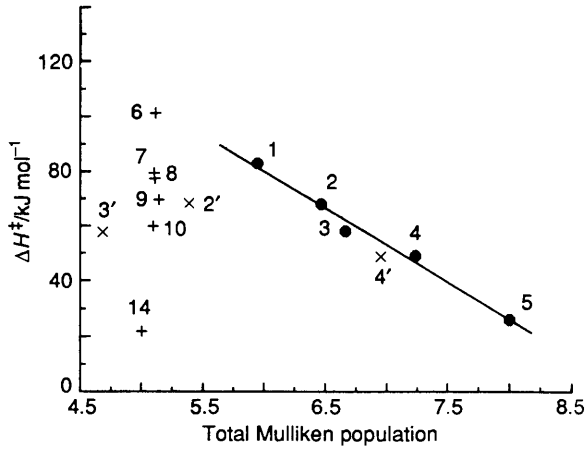

Fig. 5 Dependence of $\Delta H^{\ddagger}$ for k_4 on the total Mulliken population of the donor atom. Numbering as in Fig. 3. The straight line is the least squares line through the data points 1–5

effects, insofar as these are measured by cone angles, do not play a significant role in controlling the rate of the reaction of B_{12a} with anionic ligands and this in turn suggests that electronic factors may be important.

A plot of $\Delta H^{\ddagger}$ against the total Mulliken population of the ligand donor atom is shown in Fig. 5. There is potentially some ambiguity as to the nature of the donor atom for SCN^-, $S_2O_3^{2-}$ and NO_2^-. If we assume that (*i*) from the evidence presented above, the primary donor atom in both SCN^- and $S_2O_3^{2-}$ is S, and (*ii*) that NO_2^- is O bonded, then Fig. 5 shows that there is a good linear correlation ($R^2 = 0.979$) between $\Delta H^{\ddagger}$ and Mulliken population for anionic L (the correlation with $\Delta S^{\ddagger}$ yields $R^2 = 0.982$). It is particularly noteworthy that the correlation does not break down if $S_2O_3^{2-}$ is taken to be either S or O bonded, but does break down if NO_2^- and SCN^- are taken to be N bonded. The present results suggest therefore that if an anionic ligand is able to bond through more than one type of atom, the predominant bonding will be through the atom which has the higher Mulliken population (S rather than N in SCN^-; O rather than N in NO_2^-; S rather than O in $S_2O_3^{2-}$). That the correlation of Fig. 5 supports bonding of $S_2O_3^{2-}$ through either S or O is presumably because of their similar total Mulliken populations. Fig. 5 also shows that there is no dependence of $\Delta H^{\ddagger}$ on Mulliken population for neutral N-donor ligands; the Mulliken populations of the donor atoms are virtually the same while there is considerable variation in $\Delta H^{\ddagger}$. Steric, rather than electronic effects (Fig. 4) are clearly responsible for controlling the reaction rates of these reactions.

The position of cationic histaminium in these series is unresolved. It appears to follow the trend of $\Delta H^{\ddagger}$ with Mulliken population (Fig. 5), but this is probably coincidental, and it does not fall very well into the trend of $\Delta H^{\ddagger}$ with cone angle observed for neutral N-donor ligands (Fig. 4). Histaminium may belong to a different class; the kinetics of the reaction of B_{12a} with a series of cationic ligands will be investigated and reported on elsewhere.

The present results have provided further evidence[2,42] that the mechanism of the ligand substitution reactions of cobalt corrinoids is an I_d mechanism and that the incoming ligand, L, participates in the transition state. The rate of the reaction with anionic L depends on the electron density on the donor atom and ligands which are able to form linkage isomers react predominantly through the donor atom with the higher electron density. Steric effects appear to be of secondary importance. For a series of ligands where the electron density on the donor atom is approximately the same, such as neutral N-donor ligands, steric effects appear to be responsible for controlling reaction rates.

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