Notes

Activation Parameters for the Reaction of Aquocobalamin (Vitamin B_{12a}) with Small Anionic and Neutral Ligands*

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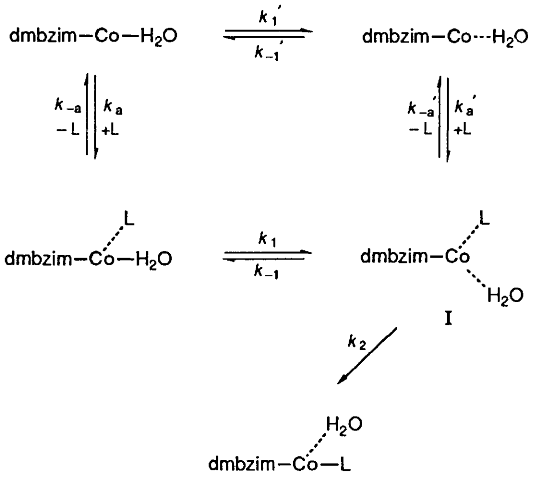
From the temperature dependence of the rate constants, activation enthalpies and entropies were determined for the reaction of aquocobalamin (vitamin B_{12a}) with SCN^-, $S_2O_3^{2-}$, NO_2^-, SO_3^{2-} and HSO_3^- (ionic strength, I = 1.0 mol dm^{-3}, KCl). These, and previously determined activation parameters for CN^-, HCN, N_3^- and HN_3, demonstrate the very small differences that exist between most small anionic and neutral ligands in their reactions with B_{12a}. Exceptions are CN^- and SO_3^{2-} which have, respectively, anomalously small and large values for $\Delta H^\ddagger$ and $\Delta S^\ddagger$. These effects are possibly due to nucleophilic participation of these ligands in the transition state of the reaction.

Aquocobalamin† (vitamin B_{12a}) contains a cobalt(III) ion co-ordinated in the equatorial plane by a corrin and in the axial positions by 5,6-dimethylbenzimidazole (dmbzim) and H_2O.[1,2] The co-ordinated water can be replaced by a wide variety of ligands in reactions which, for Co^{III}, are surprisingly fast, presumably due to the *cis* effect of the delocalised π-electron system of the corrin ring. Although the *trans* effect can profoundly influence rate constants, the following second-order rate constants (in parentheses in dm^3 mol^{-1} s^{-1}) for cobalt(III) complexes with N-donor equatorial ligands are illuminating; corrin (10^3) (for example, refs. 1–9); porphyrin (2);[10] bis(ethylenediamine) (10^{-2});[11] bis(dimethylglyoximate) (10^{-4});[12] and $(NH_3)_4$ (10^{-5}).[13] This has prompted considerable interest in the kinetics of ligand-substitution reactions of the cobalt corrinoids with B_{12a} receiving the greatest attention.

The rate of substitution of H_2O in B_{12a} by ligands such as Br^-, I^-, SCN^-, NCO^-, $S_2O_3^{2-}$, N_3^-, SO_3^{2-}, HSO_3^- and CN^- varies by about two orders of magnitude whereas the formation constants vary by about 11 orders of magnitude; this led to the view that the intimate mechanism of the substitution process was essentially dissociative (D).[3] Randall and Alberty,[5] however, pointed out that the significantly slower rate of reaction of B_{12a} with imidazole [27 dm^3 mol^{-1} s^{-1}; *cf.* (0.1–7.1) × 10^3 dm^3 mol^{-1} s^{-1} for small anionic and neutral ligands] might indicate that the reaction does not simply proceed through a unimolecular release of water followed by fast binding of the incoming ligand. Reenstra and Jencks[7] showed that a purely dissociative mechanism (upper path, Scheme 1) in a co-ordinating solvent is unlikely because of the short lifetime of the five-co-ordinate complex, **I**. All reported ligand-substitution reactions of B_{12a} are second order with rate-determining addition of the ligand L; hence a D mechanism will only be followed under the unusual circumstances where addition of water to **I** is slow compared to diffusion of L away from the outer-sphere complex (*i.e.* $k_{-1} < k_{-a}'$), but fast compared to the addition of the ligand through the k_2 step. In a non-co-ordinating solvent a D mechanism is likely to be more common because **I** will have a sufficiently long existence to allow for a diffusion-controlled encounter with a new ligand. In aqueous solution, therefore, a dissociative interchange (I_d) mechanism is likely to be operative (lower path, Scheme 1) and the rate of substitution will be influenced by any participation of bond formation between L and Co before the rupture of the Co–O bond is complete.



Scheme 1 Reaction of aquocobalamin with incoming ligand, L

The existence of nucleophilic participation by imidazole derivatives in the transition state of the ligand-substitution reactions of B_{12a} has recently been demonstrated.[1] Rate constants for reaction with small anionic and neutral ligands are available but activation parameters such as $\Delta H^\ddagger$ and $\Delta S^\ddagger$ have not been reported. I have reinvestigated the kinetics of reaction of B_{12a} with small neutral and anionic ligands (CN^-, HCN, N_3^-, HN_3, SO_3^{2-}, HSO_3^-, SCN^-, $S_2O_3^{2-}$ and NO_2^-) as a function of temperature to determine activation parameters under uniform conditions so as to establish more clearly the reasons for the reported insensitivity of the rate to the identity of the ligand, and report on the results in this Note.

Experimental

Hydroxocobalamin (> 99% pure, HPLC) was purchased from

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* *Supplementary data available* (No. SUP 56803, 4 pp.); primary kinetic data. See Instructions for Authors, *J. Chem. Soc., Dalton Trans.*, 1991, Issue 1, pp. xviii–xxii.

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

Table 1 Rate constants and activation parameters for the reaction of aquocobalamin with various ligands (ionic strength, I = 1.0 mol dm^{-3}, KCl)

Ligand, L	pH	T/°C	λ/nm[a]	$k_{11}^{\text{obs}\,b}$/dm^3 mol^{-1} s^{-1}	k_{11}^{c}/dm^3 mol^{-1} s^{-1}	$\Delta H^‡$/kJ mol^{-1}	$\Delta S^‡$/J K^{-1} mol^{-1}
SCN^-	7.07	10.0	560	300 ± 8	318 ± 8		
	7.05	15.1		461 ± 28	492 ± 30		
	6.99	25.0		1290 ± 30	1400 ± 40		
	6.99	35.1		2890 ± 60	3250 ± 70		
	6.98	45.0		5850 ± 80	6860 ± 100	63.1 ± 1.1	26 ± 4
$S_2O_3^{2-}$	7.07	10.0	500	20.1 ± 0.2	21.3 ± 0.2		
	7.04	15.1		36.2 ± 0.1	38.7 ± 0.1		
	7.00	25.0		94.9 ± 2.1	103 ± 2		
	6.99	35.0		231 ± 3	260 ± 3		
	6.98	45.0		508 ± 6	595 ± 7	68.5 ± 0.7	23 ± 2
NO_2^-	7.06	10.1	460	61.8 ± 0.2	65.4 ± 0.2		
	7.05	15.1		102 ± 0.(3)	109 ± 0.(4)		
	7.00	24.8		264 ± 2	287 ± 2		
	6.99	34.8		597 ± 9	672 ± 10		
	6.97	45.1		1260 ± 30	1470 ± 40	67.1 ± 1.0	26 ± 3
SO_3^{2-}	9.51	20.2	535	0.0842 ± 0.0049	1.88 ± 0.11		
	9.40	24.9		0.154 ± 0.004	3.26 ± 0.08		
	9.18	34.9		0.615 ± 0.009	11.4 ± 0.2		
	9.01	42.7		1.32 ± 0.06	21.8 ± 1.0		
	8.87	49.0		2.53 ± 0.13	38.4 ± 2.0		
	8.76	54.1		4.52 ± 0.28	62.6 ± 3.9	79.9 ± 0.5	33 ± 1
HSO_3^-	3.58	11.2	555	27.3 ± 0.1	27.3 ± 0.1[d]		
	3.56	20.2		71.1 ± 0.3	71.1 ± 0.3		
	3.55	25.0		114 ± 0.(5)	114 ± 0.(5)		
	3.53	35.0		292 ± 2	292 ± 2		
	3.49	49.2		953 ± 8	953 ± 8	67.2 ± 0.4	20 ± 1
CN^-						51.7 ± 1.1	−25 ± 4[e]
HCN						72.0 ± 1.8	50 ± 1[e]
N_3^-						65.9 ± 0.7	28 ± 2[f]
HN_3						69.8 ± 1.3	27 ± 4[f]

[a] Monitoring wavelength. [b] Second-order rate constant determined from the slop of k^{obs} *vs.* [L]. [c] Second-order rate constant corrected for the fraction of inert hydroxocobalamin in solution (see text). [d] No correction required since [hydroxocobalamin] ≈ 0. [e] Ref. 9. [f] Ref. 15.

Roussel. All other reagents were of analytical grade. Doubly distilled water was purified by passage through a Millipore MilliQ system (18 MΩ cm). The displacement of H_2O by L according to equation (1) was studied spectroscopically using a Cary 2300 spectrometer with [B_{12a}] = 80 μmol dm^{-3} and [L] =

$$\text{dmbzim–Co–H}_2\text{O} + \text{L} \underset{k_r}{\overset{k_{11}}{\rightleftharpoons}} \text{dmbzim–Co–L} + \text{H}_2\text{O} \quad (1)$$

0.1 mol dm^{-3} at an appropriate pH, *viz.* 7 for NO_2^-, SCN^- and $S_2O_3^{2-}$, 9.5 for SO_3^{2-} and 3.5 for HSO_3^- (the pK_a values of H_2SO_3 are [14] 1.81 and 6.91) and a suitable wavelength was chosen for following the kinetics of the reaction (Table 1).

Kinetic data were acquired with a Hi-Tech Scientific SF-51 stopped-flow spectrometer interfaced through a DAS-50 A/D converter with a computer. Pseudo-first-order rate constants, k^{obs}, were obtained by curve-fitting the absorbance–time traces using a non-linear least-squares program employing a Newton–Raphson procedure. The temperature was maintained constant (± 0.1 °C) with a circulating thermostatted water-bath. A ligand solution at the desired concentration and pH (acetate, pH 3.5; phosphate, pH 7.0; Tris[2-amino-2-hydroxymethylpropane-1,3-diol]–HCl, pH 9.5 buffers, all 0.1 mol dm^{-3}, ionic strength, I = 1.0 mol dm^{-3} with KCl) was mixed with an equal volume of a solution of B_{12a} (to a final concentration of 1.2×10^{-4} mol dm^{-3}) at the same pH and ionic strength using the stopped-flow apparatus and the absorbance changes were recorded for at least six half-lives. A range of ligand concentrations (always at least 40 times that of the B_{12a} concentration) was used and the observed second-order rate constant, k_{11}^{obs}, was determined from the slopes and intercepts of plots of $\ln(k_{11}h/k_BT)$ against $1/T$ where h and k_B are the Planck and Boltzmann constants, respectively. The pH of solutions was measured with a Metrohm 605 pH meter calibrated against standard buffers.

Results and Discussion

Aquocobalamin reacts with N_3^- and HN_3,[15] SO_3^{2-}, HSO_3^-, $S_2O_3^{2-}$, NO_2^- and SCN^- according to equation (1) to form a 1:1 adduct; in the case of CN^- there is a subsequent much slower reaction where dmbzim is displaced by a second ligand from solution.[7,9] However, the first reaction can be followed unambiguously by monitoring the absorbance changes at an isosbestic point for the second reaction. Both anions and their conjugate acids (*e.g.* CN^- and HCN; N_3^- and HN_3) act as nucleophiles towards Co^{III}.[7,9,15] Hydroxocobalamin (pK_a of co-ordinated H_2O is 8.10 at 25 °C, ΔH = 28.6 ± 0.3 kJ mol^{-1}, ΔS = −59.0 ± 1.2 J K^{-1} mol^{-1} at 1.0 mol dm^{-3} ionic strength[9]) is inert to substitution.[7,9,15,16] At pH 7, solutions of thiocyanate, thiosulphate, and nitrite contain virtually exclusively the free anion. In the case of sulphite, at pH 3.5 the amount of both H_2SO_3 and SO_3^{2-} in solution is negligible, while at pH 9.5 only SO_3^{2-} occurs in solution to any appreciable extent. Hence, by suitable choice of pH, the observed second-order rate constant can be converted into a pH-independent second-order rate constant, k_{11}, according to equation (2), where

$$k_{11} = k_{11}^{\text{obs}}(1 + K_{\text{Co}}/[\text{H}^+]) \qquad (2)$$

pK_{Co} is the acid dissociation constant for co-ordinated H_2O in B_{12a} (corrected for its temperature variation). The values of k_{11}^{obs}, k_{11}, $\Delta H^‡$, and $\Delta S^‡$ are listed in Table 1 together with previously determined activation parameters for CN^-, HCN,[9] N_3^- and HN_3.[15] Although it was observed spectroscopically that HNO_2 reacts with B_{12a}, HNO_2 has a limited solubility in

Table 2 Second-order rate constants and stability constants for the reaction of aquocobalamin with small anionic and neutral ligands

Ligand, L	$k_{25\,°\mathrm{C}}{}^{a}$/dm^3 mol^{-1} s^{-1}	log K^b	Ref.
CN^-	270	14.1	17
HCN	10.4		
N_3^-	514	4.9	18
HN_3	94.5		
SCN^-	1250	3.1	19
$S_2O_3^{2-}$	98.8	3.9	19
NO_2	293	5.4	18
SO_3^{2-}	3.31	7.3	18
HSO_3^-	116	$<3.5^c$	

^{a} Determined from the activation parameters of Table 1. ^{b} From the reference quoted in the last column, for the reaction dmbzim–Co–H_2O + L $\rightleftharpoons$ dmbzim–Co–L + H_2O. ^{c} From the observation that the pK_a of co-ordinated HSO_3^- is <3 (see text), the pK_a of free HSO_3^-, and log K for the binding of SO_3^{2-}.

water; this led to bubble formation on the cell windows and no meaningful kinetic data could be obtained.

The observation (Table 2) that there is no correlation between the rate of substitution of H_2O in B_{12a} and the stability constant of dmbzim–Co–L is not sufficient cause to exclude nucleophilic participation of these ligands in the transition state. For example, it has recently been established[1] that the rate of substitution of co-ordinated water in aquocobalamin varies linearly with the base strength of an incoming derivative of imidazole, but that the base strength of the ligand plays virtually no role in determining the stability of the complex.[20] A better appreciation of the factors controlling the reaction rates is gained by examination of the activation parameters (Table 1). For N_3^-, HN_3, SCN^-, $S_2O_3^{2-}$, NO_2^- and HSO_3^- there is little variation in either $\Delta H^‡$ (63–70 kJ mol^{-1}) or $\Delta S^‡$(20–28 J K^{-1} mol^{-1}). What is noteworthy is (*a*) the significantly smaller $\Delta H^‡$ and markedly negative $\Delta S^‡$ for the reaction with CN^-, (*b*) the large $\Delta H^‡$ and larger than average $\Delta S^‡$ for the reaction with SO_3^{2-}, and (*c*) that the reaction with HCN has a somewhat higher than average $\Delta H^‡$ and smaller than average $\Delta S^‡$.

The low $\Delta H^‡$ value for the reaction with CN^- strongly suggests that bond formation between Co and CN^- in the transition state compensates for cleavage of the Co–O bond, and this is consistent with the markedly negative $\Delta S^‡$ for this process. Hydrogen cyanide is a far weaker nucleophile than CN^- (CN^- is more basic than HCN towards the proton by over 11 orders of magnitude[21]) and a $\Delta H^‡$ value of 72 kJ mol^{-1} for this species reflects minimal, if any, Co–N bond formation in the transition state. This in turn suggests that for ligands such as N_3^-, HN_3, $S_2O_3^{2-}$, $HSO^-{}_3$, NO_2^- and SCN^- ($\Delta H^‡$ = 63–70 kJ mol^{-1}) the extent of participation in the transition state is very small.

The large $\Delta H^‡$ for reaction with SO_3^{2-} is noteworthy. Sulphite is known to have a strong *trans*-labilising effect in cobalamin complexes[2,22] and behaves, in this respect, not unlike an alkyl ligand. The values deduced by Pratt[2] for the labilisation of dmbzim by the *trans* ligand, L [equation (3)], are

$$\text{dmbzim}\ldots\text{Co–L} \xrightleftharpoons{K_{5/6}} \text{dmbzim–Co–L} \qquad (3)$$

illuminating. (The dotted line represents dmbzim bonded to the corrin ring through the nucleotide side-chain but not co-ordinated to the metal ion.) For L = CN^-, SO_3^{2-}, and CH_3 $\log(K_{5/6}) = >6.3$, 2.7, and 2.0, respectively. The ground state of sulphitocobalamin therefore has a measurable contribution from the five-co-ordinate complex, *i.e.* there is considerable weakening of the Co–N bond. This observation then provides an explanation for the observed activation parameters. The large value of $\Delta H^‡$ arises from weakening of the Co–N bond as the Co–SO_3^{2-} bond is formed in the transition state; this is also reflected in the larger than average $\Delta S^‡$ value for the process. Analogous effects are not seen with HSO_3^-, presumably because it is less polarisable. It was observed that the spectrum of sulphitocobalamin at 25 °C is virtually the same at pH 3 and 9.5, *i.e.* the pK_a of co-ordinated HSO_3^- is <3 (*cf.* 6.91 for the free ligand). The observation that the activation parameters for the reaction of HSO_3^- are similar to those of other ligands and markedly different to those for reaction with SO_3^{2-} then suggests that deprotonation occurs after the transition state. The same conclusion is applicable to the reaction with HCN which undergoes deprotonation subsequent to co-ordination and eventual isomerisation to the C-bound species.[7,9]

Of ligands studied, only SCN^- and SO_3^{2-} gave significantly non-zero intercepts for plots of k^{obs} against ligand concentration. From the intercepts, k_r for SCN^- = 0.9 s^{-1} at 25 °C, $\Delta H^‡$ = 89 ± 4 kJ mol^{-1}, $\Delta S^‡$ = 52 ± 12 J K^{-1} mol^{-1}, and k_r for SO_3^{2-} = 4 × 10^{-3} s^{-1} at 25 °C, $\Delta H^‡$ = 96 ± 7 kJ mol^{-1}, and $\Delta S^‡$ = 32 ± 22 J K^{-1} mol^{-1}. The ambident nucleophilicity of SCN^- may be responsible for a measurable reverse rate constant; it is known[23] that the initially N-bound species subsequently equilibrates with the S-bound species. A similar effect may be operative in the reaction with sulphite.

It would appear that in at least two cases of small anionic ligands, *viz.* CN^- and SO_3^{2-}, and significantly ligands which have large log K values (Table 2), there is evidence for their participation in the transition state of the substitution reactions of B_{12a}. In the cases of the other ligands studied, such participation is likely to be small and differences which exist among them may reflect small differences in their stabilisation of the transition state of the reaction.

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