Kinetics of Reaction of Aquacobalamin with Azide Ion and Hydrazoic Acid: pH Profile and Activation Parameters[†]

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The kinetics of reaction of aquacobalamin (vitamin B_{12a}) with azide anion and hydrazoic acid, producing azidocobalamin, has been investigated as a function of ligand concentration, pH, temperature, and pressure, by stopped-flow spectrophotometry. The pH-independent rate constants (25 °C, ionic strength $I = 1.0$ mol dm^{-3}) and activation parameters are $k_1 = 525 \pm 14$ dm^3 mol^{-1} s^{-1}, $\Delta H^{\ddagger}_1 = 65 \pm 1$ kJ mol^{-1}, $\Delta S^{\ddagger}_1 = 26 \pm 2$ J K^{-1} mol^{-1}, and $\Delta V^{\ddagger}_1 = 6.4 \pm 0.1$ cm^3 mol^{-1} for the reaction with N_3^-, and $k_2 = 103 \pm 13$ dm^3 mol^{-1} s^{-1}, $\Delta H^{\ddagger}_2 = 65 \pm 3$ kJ mol^{-1}, $\Delta S^{\ddagger}_2 = 13 \pm 8$ J K^{-1} mol^{-1}, and $\Delta V^{\ddagger}_2 = 5.5 \pm 0.3$ cm^3 mol^{-1} for the reaction with HN_3. The experimental data are considered in terms of both a limiting dissociative and a dissociative interchange mechanism and it is concluded that they cannot be used to distinguish between the two possible mechanistic pathways.

Aquacobalamin[‡] (vitamin B_{12a}) contains a water molecule in the sixth co-ordination position of Co^{III} *trans* to the nitrogen-containing base 5,6-dimethylbenzimidazole (dmbzim). There has been considerable interest[1–9] in the kinetics and thermodynamics of the ligand substitution reactions of aquacobalamin since co-ordinated H_2O is readily replaced by a wide variety of ligands, L, in reactions which, for low-spin Co^{III}, are surprisingly fast. These reactions have been the subject of a number of recent reports,[10–15] mostly dealing with the reaction kinetics.

The mechanism of ligand substitution in vitamin B_{12a} is generally accepted to be dissociative since the rate of anation of B_{12a} by a variety of ligands varies only by about two orders of magnitude, whereas the stability constants vary by about eleven orders of magnitude.[5,6] It has been suggested that the identity of the incoming ligand does not play an important role in the rate of the ligand substitution reactions of B_{12a},[4] and support for this has recently been provided[14] with the demonstration that the enthalpies and entropies of activation for small anionic ligands such as SCN^-, $S_2O_3^{2-}$, NO_2^- and HSO_3^- are all similar (*viz.* 63–70 kJ mol^{-1} and 20–28 J K^{-1} mol^{-1}, respectively).

Less uniformity in agreement has been achieved in the case of larger incoming ligands, however. Randall and Alberty[2,3] stated that the kinetics of the system cannot be accounted for in terms of a rate-limiting, unimolecular loss of water followed by rapid binding of the ligand in the case of the reaction with imidazole, for example, since it is substantially slower than that observed with small ligands such as SCN^-. Reenstra and Jencks[8] held that in a co-ordinating solvent such as water a purely D mechanism is untenable and suggested that the reactions are best described as being I_d, allowing for participation, however small, of the incoming ligand in the transition state of the reaction. An extension[10] of their work demonstrated that both CN^- and HCN act as nucleophiles towards the metal ion in B_{12a}, that the transition state for substitution by CN^- is enthalpically stabilised by more than 20 kJ mol^{-1} relative to that for HCN substitution, and that $\Delta S^{\ddagger}$ for the former is -25 J K^{-1} mol^{-1} but $+50$ J K^{-1} mol^{-1} for the latter, so that the apparent similarity in rate constants at 25 °C is due to a compensating variation in these two parameters.

The rate of substitution of H_2O by a series of imidazole derivatives varies linearly with the pK_a of the ligand;[11] the reaction with SO_3^{2-} has anomalously large $\Delta H^{\ddagger}$ (80 kJ mol^{-1}) and $\Delta S^{\ddagger}$ (33 J K^{-1} mol^{-1}) values;[14] there is a compensatory variation in $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ in the reactions with primary amines, with $\Delta H^{\ddagger}$ varying between about 80 and 50 kJ mol^{-1} as $\Delta S^{\ddagger}$ varies between about 50 and -50 J K^{-1} mol^{-1}, and the rate of substitution by these amines varies linearly with their donor power.[15] These effects have all been attributed to the participation of the incoming ligand in the transition state of the reaction, hence supporting an I_d mechanism.

One way of providing further information on this question is to determine the volume of activation of a reaction from the pressure response of the rate constant.[16,17] As part of an investigation into the kinetics of the reaction of aquacobalamin with azide, we have performed a pressure-dependence study of the rate constants under conditions where the ligand exists virtually exclusively in solution as either HN_3 (pH 2.5) or N_3^- (pH 6.5). While this work was in progress a report[12] appeared in which activation parameters for the reaction of azide with aquacobalamin in unbuffered aqueous solution (*ca.* pH 6.5) and 0.5 mol dm^{-3} ionic strength were reported. The full pH profile and the pressure measurements performed at low pH reported in this paper allow for a more complete discussion of the details of the mechanism of this reaction.

Experimental

Hydroxocobalamin (>99% pure, HPLC) was purchased from Roussel. All other reagents were of analytical grade. In preliminary experiments the displacement of H_2O by HN_3/N_3^- [equation (1), where only the axial ligands are shown, and the

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

[†] *Supplementary data available* (No. SUP 56843, 10 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 Second-order rate constants for the reaction of HN_3 and N_3^- with B_{12a} (I = 1.00 mol dm^{-3}, KCl)

pH	T/°C	p/MPa	k_{II}^{obs}/dm^3 mol^{-1} s^{-1}
2.00	15	Ambient	29(1)
2.44			35.9(9)
3.00			37.4(7)
3.53			60.9(3)
4.00			77.3(7)
5.10			177(5)
5.78			192(2)
7.08			198(2)
8.00			135(4)
9.21			11.5(5)
2.00	25	Ambient	85(7)
2.44			103(2)
3.00			110(3)
3.53			177(2)
4.00			197(3)
4.55			385(2)
5.10			421(2)
5.78			491(3)
6.52			532(5)
7.08			489(6)
8.00			246(12)
9.21			34(2)
2.50		5	120(2)
		25	117(1)
		50	112(1)
		75	105(1)
		100	98.7(6)
6.50		5	532(15)
		25	504(15)
		50	474(14)
		75	445(17)
		100	415(17)
2.00	35	Ambient	180(23)
2.44			254(5)
3.00			281(12)
3.53			418(3)
4.00			516(3)
5.10			1095(5)
5.78			1302(28)
7.08			1148(12)
8.00			547(5)
9.21			104(4)

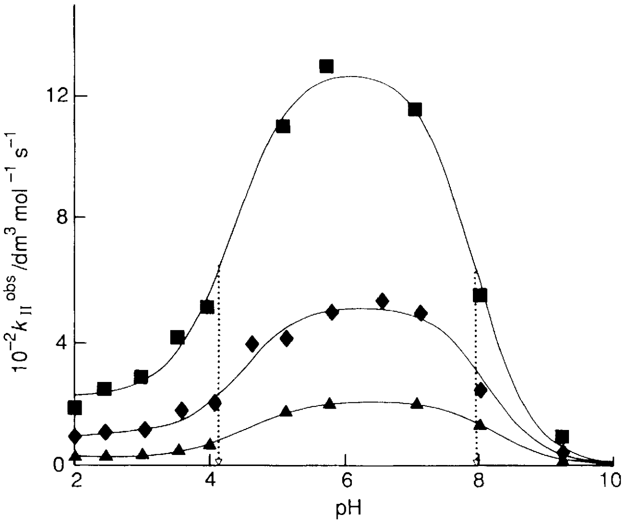

Fig. 1 The pH profile for the observed second-order rate constant, k_{II}^{obs}, of the reaction of B_{12a} with azide and hydrazoic acid at 35 (■), 25 (◆), and 15 °C (▲). The solid lines are non-linear least-squares fits to the data using equation (4) of the text

overall charge is neglected for convenience] was studied spectroscopically using either a Cary 2300 or a Philips PU 8700 spectrometer with [B_{12a}] = 47.6 µmol dm^{-3} (standardised by conversion into dicyanocobinamide, for which ε_{367} = 3040 m^2 mol^{-1})[18] and [azide] = 0.1 mol dm^{-3} at pH 7 to establish the appropriate wavelengths for the subsequent stopped-flow and high-pressure stopped-flow measurements.

The kinetics of the reaction was followed using solutions with [B_{12a}] = 50 µmol dm^{-3}, 1 $\leqslant$ [azide] $\leqslant$ 100 mmol dm^{-3} and 2 $\leqslant$ pH $\leqslant$ 9. These were prepared by adding an aliquot of a standardised stock solution of hydroxocobalamin to and dissolving a weighed amount of sodium azide in, respectively, the analytically required amount of H_3PO_4–KH_2PO_4 or tris(hydroxymethyl)aminomethane–HCl buffer and subsequently mixing the complex and ligand solutions in a 1 : 1 ratio in the stopped-flow spectrometer. The reported pH is the mean for the five separate solutions of different ligand concentration. The pH was measured with a DDS 1100 pH meter and Metrohm combination glass electrode calibrated against standard buffers. The final ionic strength of all solutions was adjusted to I = 1.0 mol dm^{-3} with KCl. The kinetic measurements were performed under pseudo-first-order conditions at λ = 370 (ambient pressure) and 550 nm (high pressure) using a Hi-Tech SF4 stopped-flow spectrometer interfaced with a microcomputer. Data analysis was performed using a Hi-Tech AD2 data acquisition and analysis program.

The temperature was regulated (± 0.1 °C) using an external Colora MC4 cryostat and an internal electronic temperature controller. The pressure dependence of the reaction (5 $\leqslant p \leqslant$ 100 MPa) at 25 °C (using the same solutions for the kinetic runs at pH 2.5 and 6.5 as used for the ambient-pressure measurements) was studied using a high-pressure stopped-flow cell similar to that described by van Eldik.[19] The pseudo-first-order rate constants, k_I^{obs}, were determined by fitting the absorbance–time traces to an exponential function using a non-linear least-squares routine employing a Newton–Raphson procedure. Second-order rate constants, k_{II}^{obs}, were determined from the gradient of a linear least-squares fit of a plot of k_I^{obs} *vs.* [L].

Results and Discussion

The substitution of H_2O by azide at neutral pH is readily observed spectroscopically with a shift in the band maximum of the γ, β and α bands from 350 (ε = 2210 m^2 mol^{-1}), 500, and 525 nm to 358 (ε = 1900 m^2 mol^{-1}), 515 and 540 nm, respectively. The reactions gave simple first-order kinetics under the experimental conditions used and the intercepts of plots of k_I^{obs} *vs.* [L] were never statistically different from zero. The second-order rate constants, k_{II}^{obs}, are listed in Table 1 and plotted in Fig. 1.

The kinetically determined pK_a values of HN_3 (4.1) and B_{12a} (7.9), shown by the dotted lines at the inflection points of Fig. 1, are in good agreement with values calculated from the reported enthalpies and entropies of ionisation of the two species (see below), *viz.* 4.38 and 7.93. The pH profile can be accounted for by a mechanistic scheme similar to that for the reaction of cyanide with B_{12a}.[8,10] There are two routes [equations (2) and (3)] to the formation of azidocobalamin. Both N_3^- and HN_3 can displace H_2O from Co^{III} with k_1 and k_2, respectively, being the relevant rate constants. Azidocobalamin exists as a single species in the range 2 < pH < 10 since there is no detectable change in its electronic spectrum; this means that the pK_a of co-ordinated HN_3 is < 2. (There are a number of precedents in cobalamin chemistry for lowering of the pK_a of an ionisable functional group on a ligand on co-ordination by Co^{III}).[13,20] Deprotonation of bound HN_3 must therefore occur in a subsequent, fast step. Hydroxocobalamin is assumed inert to substitution in accordance with previous reports.[1,8,10]

Table 2 Derived values of the second-order rate constants for the displacement of H_2O in B_{12a} by N_3^- and HN_3

T/°C	$k_1{}^a$/dm^3 mol^{-1} s^{-1}	$k_2{}^b$/dm^3 mol^{-1} s^{-1}
15	209 ± 4	36 ± 3
25	525 ± 14	103 ± 13
35	1304 ± 27	227 ± 21

[a] For displacement of H_2O by N_3^-. [b] For displacement of H_2O by HN_3.

Table 3 Activation parameters for the reaction of azide with aquacobalamin (ionic strength I = 1.00 mol dm^{-3})

Rate constant	$\Delta H^‡$/kJ mol^{-1}	$\Delta S^‡$/J K^{-1} mol^{-1}	$\Delta V^‡$/cm^3 mol^{-1}
k_1	65 ± 1	26 ± 2	6.4 ± 0.1
k_2	65 ± 3	13 ± 8	5.5 ± 0.3

$$\text{dmbzim–Co–H}_2\text{O} + \text{N}_3^- \xrightarrow{k_1} \text{dmbzim–Co–N}_3 \qquad (2)$$

$$\text{dmbzim–Co–H}_2\text{O} + \text{HN}_3 \xrightarrow{k_2} \text{dmbzim–Co–N}_3\text{H} \qquad (3)$$

$$\text{dmbzim–Co–N}_3\text{H} \xrightarrow{\text{fast}} \text{dmbzim–Co–N}_3 + \text{H}^+$$

The observed second-order rate constant, $k_{\text{II}}^{\text{obs}}$, is a function of k_1 and k_2 [equation (4)], where pK_{Co} and pK_{L} are the

$$k_{\text{II}}^{\text{obs}} = \frac{k_1}{(1 + K_{\text{Co}}/[\text{H}^+])(1 + [\text{H}^+]/K_{\text{L}})} + \frac{k_2}{(1 + K_{\text{Co}}/[\text{H}^+])(1 + K_{\text{L}}/[\text{H}^+])} \qquad (4)$$

(temperature-dependent) acid dissociation constants of co-ordinated H_2O in B_{12a} [8.10 at 25 °C, I = 1.00 mol dm^{-3} (KCl), ΔH = 28.6 kJ mol^{-1}, ΔS = −59 J K^{-1} mol^{-1}][10] and of free HN_3 [4.54 at 25 °C, I = 1.00 mol dm^{-3}, ΔH = 12.9 kJ mol^{-1}, ΔS = −42 J K^{-1} mol^{-1}],[21] respectively.

The $k_{\text{II}}^{\text{obs}}$ values at 15, 25 and 35 °C were fitted by equation (4) using a non-linear least-squares method, with k_1 and k_2 as adjustable parameters. The fits obtained are shown as the solid lines in Fig. 1; the values of k_1 and k_2 are given in Table 2. The satisfactory fits are evidence for the adequacy of the assumed mechanism [equations (2) and (3)]. Plots of ln (k_ih/k_BT) *vs.* T^{-1}, i = 1 or 2, where h and k_B are the Planck and Boltzmann constants, respectively, gave good straight lines (see SUP 56843); from the slopes and intercepts, entropies and enthalpies of activation were determined (Table 3).

Volumes of activation were determined from the relationship (5) (which holds for the relatively low pressures used in this

$$(\partial \ln k_i/\partial p)_T = -\Delta V^‡/RT \qquad (5)$$

study) by plotting ln k_i *vs.* p; good straight lines were obtained (see SUP 56843) and $\Delta V^‡$ values determined from their slopes are listed in Table 3.

Like CN^- and HCN, both N_3^- and HN_3 act as nucleophiles towards Co^{III}, with the former reacting about five times faster than the latter. This relatively modest difference in reactivity is due to a small difference in $\Delta S^‡$ (Table 3). This is not the case for CN^- and HCN, for which $\Delta H^‡$ = 51.7 and 72.0 kJ mol^{-1} and $\Delta S^‡$ = −25 and 50 J K^{-1} mol^{-1}, respectively.[10] The values for N_3^-, which are in good agreement with those reported by van Eldik and co-workers,[12] *viz.* $\Delta H^‡$ = 65 ± 2 kJ mol^{-1} and $\Delta S^‡$ = 30 ± 5 J K^{-1} mol^{-1}, and for HN_3 fall within the range recently reported[14] for small anionic ligands, and serve to emphasise the unusual activation parameters found for CN^-, HCN and SO_3^{2-}.

The substitution of H_2O by L in octahedral aqua complexes ordinarily proceeds through an unstable five-co-ordinate intermediate (ref. 8 and references therein). There are two possible mechanisms. In the first, the D mechanism, the reaction proceeds *via* breaking of the Co–O bond, followed by an encounter with the ligand to form the unstable intermediate I, which then leads to product formation (Scheme 1). In the second, the I_d mechanism (Scheme 2), there is initial formation of an outer-sphere complex, followed by breaking of the Co–O bond to form I.

$$\text{dmbzim–Co–H}_2\text{O} \underset{k_{-\text{d}}}{\overset{k_{\text{d}}}{\rightleftharpoons}} \{\text{dmbzim–Co, H}_2\text{O}\}$$

$$\{\text{dmbzim–Co, H}_2\text{O}\} \underset{k_{-\text{b}},\ \text{L}}{\overset{k_{\text{b}},\ +\text{L}}{\rightleftharpoons}} \underset{\text{I}}{\{\text{H}_2\text{O, dmbzim–Co, L}\}}$$

$$\underset{\text{I}}{\{\text{H}_2\text{O, dmbzim–Co, L}\}} \xrightarrow{k_{\text{p}}} \text{azidocobalamin}$$

Scheme 1

$$\text{dmbzim–Co–H}_2\text{O} \underset{k_{-\text{o}},\ -\text{L}}{\overset{k_{\text{o}},\ +\text{L}}{\rightleftharpoons}} \{\text{dmbzim–Co–H}_2\text{O, L}\}$$

$$\{\text{dmbzim–Co–H}_2\text{O, L}\} \underset{k'_{-\text{b}}}{\overset{k'_{\text{b}}}{\rightleftharpoons}} \{\text{H}_2\text{O} \cdots \text{dmbzim–Co} \cdots \text{L}\}$$

$$\underset{\text{I}}{\{\text{H}_2\text{O} \cdots \text{dmbzim–Co} \cdots \text{L}\}} \xrightarrow{k_{\text{p}}} \text{azidocobalamin}$$

Scheme 2

Application of the steady-state approximation leads to similar expressions for the second-order rate constants, k_i, in terms of the microscopic rate constants [equation (6) for the D

$$k_i = k_{\text{p}}\left(\frac{k_{\text{d}}}{k_{-\text{d}}}\right)\left(\frac{k_{\text{b}}}{k_{-\text{b}}}\right) \qquad (6)$$

$$k_i = k_{\text{p}}\left(\frac{k_{\text{o}}}{k_{-\text{o}}}\right)\left(\frac{k'_{\text{b}}}{k'_{-\text{b}}}\right) \qquad (7)$$

mechanism, (7) for the I_d mechanism]. The volume of activation for the D mechanism is given by equation (8); $\Delta V^‡(k_d)$ −

$$\begin{aligned}\Delta V^‡(k_i) &= \Delta V^‡(k_{\text{d}}) - \Delta V^‡(k_{-\text{d}}) + \Delta V^‡(k_{\text{b}}) - \Delta V^‡(k_{-\text{b}}) + \Delta V^‡(k_{\text{p}}) \\ &= \Delta V^‡(k_{\text{d}}) - \Delta V^‡(k_{-\text{d}}) + \Delta \bar{V}(K_{\text{b}}) + \Delta V^‡(k_{\text{p}}) \qquad (8)\end{aligned}$$

$\Delta V^‡(k_{-d})$ should be positive and independent of the identity of the incoming ligand, L. The term $\Delta \bar{V}(K_b)$ is expected to be positive due to the addition of another species to the outer co-ordination sphere. Although $\Delta V^‡(k_p)$ should be negative for both N_3^- and HN_3, its contribution is expected to be outweighed by the two other terms, resulting in overall positive values for $\Delta V^‡$ in agreement with experimental values of Table 3. The value of $\Delta V^‡(k_p)$ should depend on the charge of the ligand, being less negative for N_3^- because of the relief of electrostriction on complexation of the anion. This, too, is in accord with the observation (Table 3) that $\Delta V^‡$ for N_3^- is more positive than for HN_3. The value of $\Delta V^‡$ for HN_3 fits well with existing data[12] for the reaction of differently charged species with aquacobalamin, *viz.* +16, +(8–9), +7, and now +5.5 cm^3 mol^{-1} for a 4−, 2−, 1−, and neutral incoming ligand, respectively. The data are therefore consistent with a D character for the substitution process.

The $\Delta H^‡$ values are identical for the reaction of the two ligands with B_{12a}. This, too, would be in accord with a limiting D mechanism where the predominant contribution to this parameter is the breaking of the Co–O bond (the k_d step).

The difference which does arise in the two rate constants

(525 ± 14 dm^3 mol^{-1} s^{-1} for N_3^- and 103 ± 13 dm^3 mol^{-1} s^{-1} for HN_3, at 25 °C) is due to a difference in $\Delta S^\ddagger$ 26 ± 2 and 13 ± 8 J K^{-1} mol^{-1}, respectively. As with $\Delta V^\ddagger(k_i)$, $\Delta S^\ddagger(k_i)$ is an overall entropy of activation [equation (9)] made up of

$$\begin{aligned}\Delta S^\ddagger(k_i) &= \Delta S^\ddagger(k_d) - \Delta S^\ddagger(k_{-d}) + \Delta S^\ddagger(k_b) - \Delta S^\ddagger(k_{-b}) \\ &\qquad + \Delta S^\ddagger(k_p) \quad (9) \\ &= \Delta S^\ddagger(k_d) - \Delta S^\ddagger(k_{-d}) + \Delta\bar{S}(K_b) + \Delta S^\ddagger(k_p)\end{aligned}$$

contributions from the individual steps of the mechanism (Scheme 1). For a D mechanism the first two terms should be identical for the two ligands; $\Delta\bar{S}(K_b)$ will be a combination of a positive contribution due to loss of solvational water from the ligand (which is expected to be greater for N_3^- than for HN_3) and from B_{12a}, and negative due to loss of freedom of the ligand (expected to be about the same for both ligands). The difference in $\Delta S^\ddagger$, which is more positive for the reaction with N_3^-, is thus attributed to release of solvational water molecules on formation of the intermediate I (Scheme 1).

It would, therefore, appear that the three activation paramerers determined are adequately explained by a limiting D mechanism. What needs to be verified, however, is whether the data can also be explained by an I_d mechanism. For an I_d mechanism equation (10) is applicable. The first term is

$$\begin{aligned}\Delta V^\ddagger(k_i) &= \Delta V^\ddagger(k_o) - \Delta V^\ddagger(k_{-o}) + \Delta V^\ddagger(k'_b) - \Delta V^\ddagger(k'_{-b}) \\ &\qquad + \Delta V^\ddagger(k_p) \quad (10) \\ &= \Delta\bar{V}(K_o) + \Delta V^\ddagger(k'_b) - \Delta V^\ddagger(k'_{-b}) + \Delta V^\ddagger(k_p)\end{aligned}$$

expected to be strongly dependent upon the charge on the incoming ligand L, the next two terms should be independent of the nature of L, and the last term, to a first approximation, should also be independent of L. Hence, diffences in $\Delta V^\ddagger$ should primarily be due to differences in ligand charge, manifest during formation of the outer-sphere complex. Hence, equation (11) is

$$\Delta(\Delta V^\ddagger)(k_i) - \Delta V^\ddagger(k_p) = \Delta\bar{V}(K_o) \qquad (11)$$

obtained. If we make the reasonable assumption that for a neutral entering ligand $\Delta\bar{V}(K_o) \approx 0$, then $\Delta V^\ddagger(k_p) \approx 5.5$ cm^3 mol^{-1}, which means that $\Delta\bar{V}(K_o) \approx 0.9$ cm^3 mol^{-1} for the reaction with N_3^-.

The volume change which occurs on forming an outer-sphere complex can be evaluated by means of equation (12) where r is

$$\Delta\bar{V} = \frac{-Z_A Z_B e^2 L}{Dr}\left[\left(\frac{\partial \ln r}{\partial p}\right)_T + \left(\frac{\partial \ln D}{\partial p}\right)_T\right] \qquad (12)$$

the critical distance (the distance of closest approach of the ions), D is the solvent permittivity, L is the Avogadro constant, and the other symbols have the usual meaning.[22] The two terms in square brackets may be rewritten in terms of the compressibility β and density ρ, of the solvent,[23] thereby reducing equation (12) to (13), where $\Delta\bar{V}$ is in cm^3 mol^{-1} and r is in Å.

$$\Delta\bar{V} = -10.7 Z_A Z_B / r \qquad (13)$$

To take ionic strength into account, $1/r$ is replaced by $(1/r)\exp(-r/r_D)$,[24] where r_D, the shielding length, is given by $(\varepsilon RT/2\rho L^2 e^2 I)^{\frac{1}{2}}$. Taking $r = 5$ Å, $\Delta\bar{V}(K_o)$ is predicted to be 0.8 cm^3 mol^{-1} with $Z_A Z_B = -2$. We have taken the charge at the metal centre to be +2; this is due to (*i*) the +3 charge on the metal ion; (*ii*) the −1 charge on the corrin ring N-donor set, and (*iii*) considering the −1 charge on the phosphate group of the nucleotide side-chain of B_{12a} to be too far away to influence matters at the reaction centre (an examination of cobalamin structures using molecular graphics shows the cobalt–phosphate distance to be close to 10 Å). Taking into account all the assumptions which had to be made, a predicted increase of 0.8 cm^3 mol^{-1} in $\Delta V^\ddagger$ on going from HN_3 to N_3^- compares remarkably well with the experimentally determined value of 0.9 cm^3 mol^{-1}.

In their analysis of the mechanism of the reaction of B_{12a} with azide and cyanoferrates, van Eldik and co-workers[12] dismissed the I_d mechanism on the basis of similar calculations. For charges of −1, −2 and −4 on the incoming ligand, $\Delta\bar{V}(K_o)$ was predicted to be 2.2, 3.3 and 5.5 cm^3 mol^{-1}, respectively, but the experimental values were approximately 3, 5 and 12 cm^3 mol^{-1}. However, these calculations were based on the assumption of a spherical B_{12a} molecule with a net charge of +1. As noted above, the phosphate group is in our opinion too remote from the reaction centre to influence matters. If their calculations are redone but with a +2 charge for B_{12a}, then the predicted values of $\Delta\bar{V}(K_o)$ are 3.3, 5.5 and 10 cm^3 mol^{-1} respectively, in very good agreement indeed with their experimental data.

Arguments analogous to those advanced for the D mechanism can be invoked to explain the lack of difference in $\Delta H^\ddagger$ and the increase in $\Delta S^\ddagger$ on changing L from HN_3 to N_3^-. Hence, on the basis of the measurement of activation parameters alone, we conclude that the mechanism for the reaction of aquacobalamin with azide and hydrazoic acid cannot be distinguished between an I_d and a limiting D mechanism.

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