Ligand-Substitution Reactions of Aquacobalamin (Vitamin B_{12a}) Revisited. Conclusive Evidence for the Operation of a Dissociative Interchange Mechanism

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A detailed kinetic study of the substitution of aquacobalamin by thiourea and substituted thiourea was performed as a function of entering ligand concentration, temperature, and pressure. The observed rate and activation parameters for the forward and reverse reaction steps conclusively confirm the operation of a dissociative interchange mechanism. For instance, the volumes of activation for both the forward and reverse reactions have values between +6 and +10 $cm^3 mol^{-1}$, accompanied by positive values for the entropy of activation. The conclusions reached in an earlier study dealing with the substitution by pyridine are in error and are reinterpreted in the light of the new data. The results are compared to all available literature information, which confirms the mechanistic assignment made in this study.

Introduction

Our group has over the past decade been most intensively involved with the application of high-pressure kinetic techniques in mechanistic studies of a large variety of inorganic, organometallic, and bioinorganic reactions.[1-5] Activation volumes obtained from the pressure dependence of rate constants exhibit a strong mechanistic discrimination ability and have greatly assisted the elucidation of the underlying reaction mechanisms. We demonstrated that, in the case of nonsymmetrical reactions,[4] it is essential to construct an overall reaction volume profile from activation and reaction volume data in order to be able to describe the mechanistic pathway in terms of volume changes along the reaction coordinate.

We and others[6-8] have recently applied this approach to ligand substitution reactions of aquacobalamin (vitamin B_{12a}), to supplement earlier ambient-pressure kinetic information[9-13] on which basis a differentiation between the operation of a dissociative interchange (I_d) or limiting dissociative (D) mechanism was not possible. In the first study[6] the effect of pressure on the anation of aquacobalamin (hereafter referred to as B_{12}–H_2O^+) by azide and several cyanoferrates favored the operation of a D instead of I_d mechanism. The interpretation of the data was complicated to some extent by solvational contributions arising from charge neutralization during bond formation with anionic ligands. In a subsequent study[8] it was found that the volumes of activation for substitution by azide and hydrazoic acid are indeed very similar and the authors concluded that these activation parameters cannot be used to distinguish between the two possible mechanistic pathways (I_d or D). In an effort to resolve this difficulty we[7] performed a detailed study of the substitution of B_{12}–H_2O^+ by a neutral ligand, viz. pyridine, and constructed a volume profile from the activation volume data for both the forward and reverse reactions. Surprisingly, the rate constant for the complex formation reaction reached a limiting value at high pyridine concentrations, and the reverse aquation reaction exhibited a remarkably positive activation volume of +17 $cm^3 mol^{-1}$. These observations led us to believe that both reactions proceeded according to a limiting D mechanism, and the volume profile was interpreted accordingly.[7]

In the meantime, we have investigated this reaction for another series of neutral ligands, viz. thiourea and substituted thiourea, and have come to the conclusion that our earlier assignment of a limiting D mechanism[7] is in error. In fact, we now report very conclusive evidence for the operation of a dissociative interchange mechanism and can demonstrate that the activation parameters do allow a nonequivocal mechanistic assignment.

Experimental Section

Materials. Crystalline hydroxocobalamin hydrochloride (Fluka) was used as the source of aquacobalamin. Thiourea was obtained from E. Merck, and the substituted derivatives were obtained from Aldrich. All other chemicals were of analytical reagent grade. All solutions were prepared with Millipore water, stored in the dark at ca. 4 °C, and used within 24 h after preparation.

Procedures and Instrumentation. The ligand solution was mixed with a B_{12}–H_2O^+ solution of the same pH and ionic strength (0.1 M). In all cases the ligand concentration was at least 10 times higher than that of B_{12}–H_2O^+. The concentration of B_{12}–H_2O^+ was kept at (0.70–1.5) × 10^{-4} M. The pH of both solutions was adjusted by addition of NaOH or $HClO_4$. pH measurements were performed on a Metrohm E250 pH meter.

UV–vis absorption spectra were recorded on a Cary 1 spectrophotometer at ambient pressure and on a Zeiss DMR 10 spectrophotometer, equipped with thermostated high-pressure cell,[14] at pressures up to 150 MPa. Kinetic measurements at ambient pressure were performed on a Durrum D110 stopped-flow spectrometer and on a three-component Biologic MPS-51 stopped-flow unit. Kinetic measurements at elevated pressure (≤150 MPa) were performed on a homemade high-pressure stopped-flow unit.[15] All instruments were thermostated to within ±0.1 °C.

Absorbance–time traces were analyzed with the aid of two data acquisition systems, viz. an IBM compatible computer using Biologic V3.23 and OLIS software and an Apple IIe system described elsewhere.[16] All reactions exhibited excellent pseudo-first-order behavior, and the

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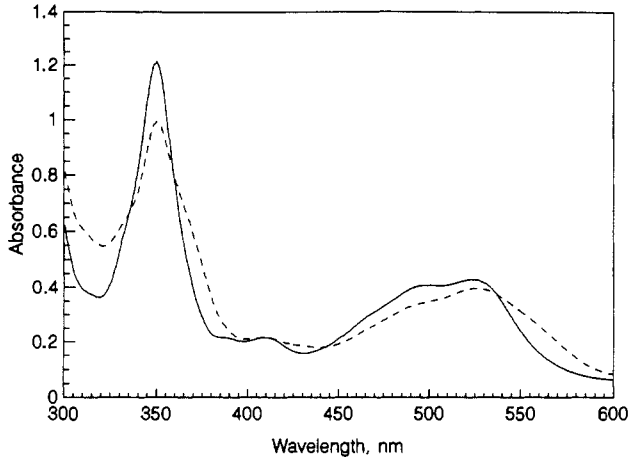

Figure 1. UV–vis spectral changes observed during the reaction of B_{12}–H_2O^+ with thiourea. Experimental conditions: $[B_{12}] = 0.70 \times 10^{-4}$ M; [TU] = 0.050 M; pH = 4.0; ionic strength = 0.10 M; optical path length = 0.88 cm; temperature = 25 °C; — before mixing, - - - after mixing in a tandem cuvette.

corresponding rate plots were linear for at least 3 half-lives of the reaction. The quoted errors of all the kinetic parameters are the standard deviations of the corresponding mean values. Kinetic traces at ambient pressure were recorded at 570 nm and at elevated pressure at 370 nm where large changes in absorbance occur during the reaction.

Results and Discussion

The reaction of B_{12}–H_2O^+ with thiourea (TU) is accompanied by characteristic spectral changes as illustrated in Figure 1. The spectra show isosbestic points at 335, 360, 409, 444, and 539 nm (TU). The observed spectral changes were found to depend on the concentration of TU employed and reached a maximum value at [TU] ≥ 0.5 M. These observations indicate that substitution of B_{12}–H_2O^+ by TU involves an equilibrium as shown in (1) for

$$B_{12}\text{–}H_2O^+ + L \rightleftharpoons B_{12}\text{–}L^+ + H_2O \qquad (1)$$

$$K = \frac{[B_{12}\text{–}L^+]}{[B_{12}\text{–}H_2O^+][L]} \qquad (2)$$

which the overall equilibrium constant is expressed in (2). The observed spectral changes can be used to estimate K with the aid of (3), where A_0, A, and A_∞ represent the absorbance at a particular

$$\frac{A - A_0}{[L]} = KA_\infty - KA \qquad (3)$$

wavelength associated with the species B_{12}–H_2O^+, an equilibrium mixture, and the species B_{12}–L^+, respectively. The corresponding plot of $(A - A_0)/[L]$ versus A for L = TU is shown in Figure 2 from which it follows that $K = 18.1 \pm 0.4$ M^{-1} at 25 °C, pH = 4.0, and 0.1 M ionic strength. Firth et al. reported a value of 13 M^{-1} at room temperature and pH ca. 5.[17]

Similar observations were made for *N,N'*-dimethylthiourea (DMTU), and the spectral measurements resulted in a K value of 4.6 ± 0.2 M^{-1} under conditions similar to those quoted in Figure 2. In the case of tetramethylthiourea (TMTU), no meaningful spectral changes were observed on addition of an excess of TMTU to B_{12}–H_2O^+, indicating that the value of K must be significantly smaller than for TU and DMTU.

A series of kinetic experiments were performed to investigate the pH dependence of the substitution process. The reaction of

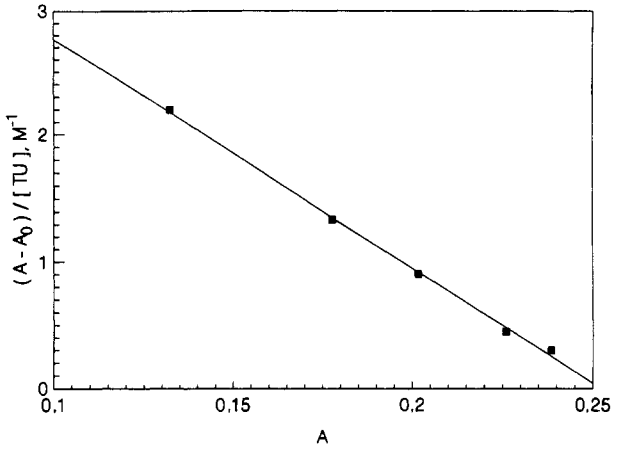

Figure 2. Plot of $(A - A_0)/[TU]$ versus A for the determination of the equilibrium constant for reaction 1 for L = TU. Experimental conditions: $[B_{12}] = 0.70 \times 10^{-4}$ M; pH = 4.1; ionic strength = 0.1 M; λ = 562 nm.

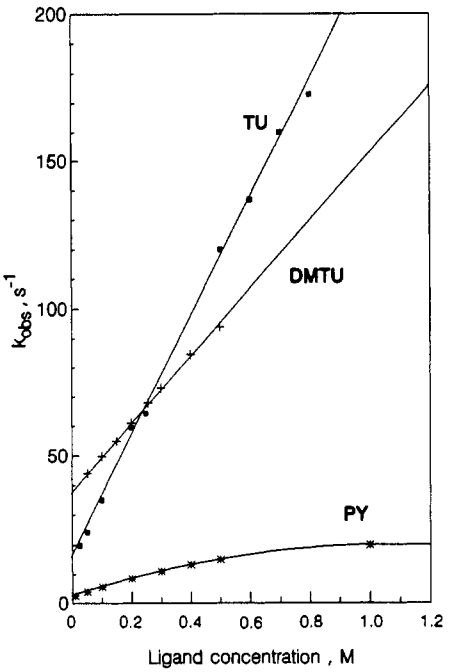

Figure 3. Plots of k_{obs} versus ligand concentration for the reaction at 25 °C B_{12}–$H_2O^+ + L \rightleftharpoons B_{12}$–$L^+ + H_2O$. Experimental conditions: For L = TU and DMTU, see Figure 4 and Table II, respectively; for L = PY, see ref 7.

B_{12}–H_2O^+ with TU exhibited a maximum, pH-independent rate constant at pH 1–5, which decreased at higher pH showing a typical sigmoid curve to almost zero at pH > 11.[8] These observations are in good agreement with the pK_a value of 8.1 reported for B_{12}–H_2O^+ in the literature[9] and demonstrate that the aqua species is the only reactive species at pH ≤ 5. Furthermore, it is known that TU and its derivatives do not protonate in this acidity range.[18] Consequently, all measurements in this study were performed at pH = 4.0.

Typical examples for the ligand concentration dependence of k_{obs} are given in Figure 3. It follows that the plots of k_{obs} versus [L] for TU and DMTU are linear in agreement with earlier observations,[19] exhibit significant intercepts, and do not indicate any saturation as observed for the reaction with pyridine (PY) before.[7] In the case of DMTU the concentration range was restricted due to viscosity problems. This behavior can be expressed by the rate law in (4), where k_a and k_b represent the

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$$k_{obs} = k_a[L] + k_b \quad (4)$$

overall rate constants for the forward and reverse reactions in (1), respectively. The values of k_a and k_b can be used to calculate K ($=k_a/k_b$), which turns out to be 13 ± 2 and 3.0 ± 0.1 M^{-1} for L = TU and DMTU, respectively. These kinetically determined K values are in reasonable agreement with those obtained from the spectral measurements under similar conditions. Furthermore, the data in Figure 3 clearly demonstrate that the limiting rate constant observed for the reaction with pyridine cannot be due to the operation of a limiting D mechanism as suggested before,[7] since it would then in principle not be possible to measure any higher rate constant for the substitution of B_{12}–H_2O^+ especially in the case of a stronger nucleophile than pyridine. Thus the nonlinear concentration dependence in the case of L = PY must be due to the operation of an I_d mechanism and involves the formation of a precursor species (see further discussion).

The substitution of B_{12}–H_2O^+ by TU was studied as a function of temperature and pressure, for which the results are reported in Figures 4 and 5, respectively. The data were fitted with eq 4, and the resulting values of k_a and k_b, along with the associated activation parameters, are summarized in Table I. Plots of ln k_a and ln k_b versus pressure are linear within the experimental error limits as shown in Figure 6. Similar data for the reaction with DMTU are summarized in Table II along with the associated activation parameters. Once again the plots of k_{obs} versus [DMTU] at various temperatures and pressures, and the plots of ln k_a and ln k_b versus pressure, are good straight lines. An important observation of this study is the fact that the reactions of B_{12}–H_2O^+ with the stronger nucleophiles TU and DMTU do not exhibit the saturation in the k_{obs} versus [L] plots as observed for the reaction with PY before[7] (see Figure 3). Furthermore, the significantly higher k_{obs} values observed for these nucleophiles as compared to the limiting rate constant reached in the pyridine case clearly demonstrates that a limiting D mechanism, for which the maximum rate constant will represent rate-determining breakage of the B_{12}–H_2O^+ bond,[7] cannot be operative. Thus the significant curvature observed for the reaction with PY is very misleading and must be interpreted in a different way. In fact, recent studies by Marques et al.[20] have confirmed the nonlinear concentration dependence of k_{obs} for the reaction with PY, and similar effects were also found for 4-methylpyridine, imidazole, methyl glycinate, and histamine. This curvatuve can only be ascribed to some precursor formation in terms of a dissociative interchange mechanism given in (5), for which the corresponding

$$B_{12}\text{–}H_2O^+ + L \rightleftharpoons [B_{12}\text{–}H_2O^+\cdot L] \quad K_1$$

$$[B_{12}\text{–}H_2O^+\cdot L] \rightleftharpoons B_{12}\text{–}L^+ + H_2O \quad k_2, k_{-2} \quad (5)$$

$$k_{obs} = \frac{k_2K_1[L]}{1 + K_1[L]} + k_{-2} \quad (6)$$

expression for k_{obs} is given in (6). At this point it is uncertain why the mentioned ligands do exhibit signifficant presursor formation and others do not (see further discussion). For L = TU and DMTU the plots of k_{obs} versus [L] are linear, indicating that $1 + K_1[L] \approx 1$, and (6) reduces to (7), which is in agreement with the empirical rate law (4), where $k_a = k_2K_1$ and $k_b = k_{-2}$.

$$k_{obs} = k_2K_1[L] + k_{-2} \quad (7)$$

A comparison of the data for k_a (i.e. k_2K_1 in (5)) found in this study with those reported for a wide series of nucleophiles in the literature[6-13] reveals some interesting trends. In all investigated reactions, with the exception of PY,[7] no significant curvature was observed in the plots of k_{obs} versus the entering ligand concentration, from which it follows that K_1 is very small, i.e. $K_1 \ll 1$. This is not surprising for the neutral ligands investigated, although larger effects are expected on electrostatic grounds for ligands such as SO_3^{2-} and $S_2O_3^{2-}$, which was not the case.[13] In this respect it is important to note that there exists some uncertainty concerning the overall charge distribution on the B_{12} molecule, whether it should be treated as a +1 charged species or as a +2 charged species where the −1 charge on the phosphate

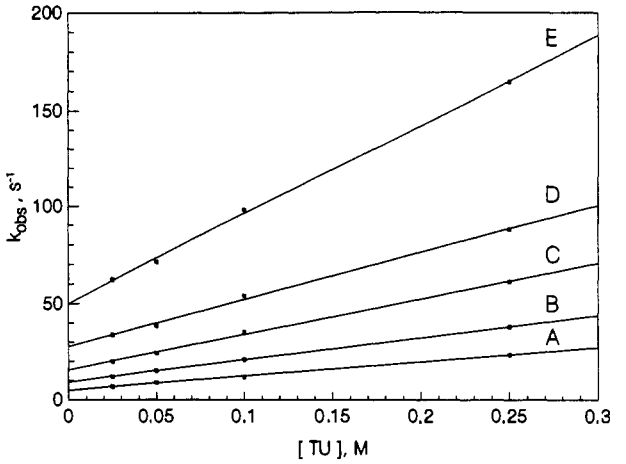

Figure 4. Plots of k_{obs} versus [TU] for reaction 1 as a function of temperature. Experimental conditions: $[B_{12}] = 0.70 \times 10^{-4}$ M; pH = 4.0; ionic strength = 0.1 M; temperature = 15.0 (A), 20.0 (B), 25.0 (C), 30.0 (D), and 35.0 (E) °C.

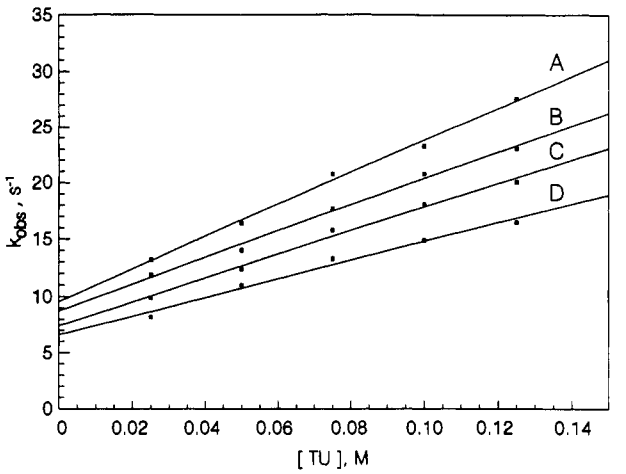

Figure 5. Plots of k_{obs} versus [TU] for reaction 1 as a function of pressure Experimental conditions: $[B_{12}] = 1.4 \times 10^{-4}$ M; pH = 4.0; ionic strength = 0.1 M; pressure = 10 (A), 50 (B), 100 (C), and 150 (D) MPa.

Table I. Rate and Activation Parameters for the Substitution of B_{12}–H_2O^+ by TU[a]

temp, °C	p, MPa	k_a, M^{-1} s^{-1}	k_b, s^{-1}
15.0	0.1	73 ± 4	4.9 ± 0.6
20.0		115.1 ± 0.8	9.2 ± 0.1
25.0		184 ± 5	15.5 ± 0.4
30.0		243 ± 10	28 ± 1
35.0		461 ± 11	50 ± 2
20.0	10	142 ± 7	9.6 ± 0.5
	50	117 ± 6	8.7 ± 0.5
	100	104 ± 6	7.4 ± 0.5
	150	82 ± 6	6.6 ± 0.5
$\Delta H^{\ddagger}$, kJ/mol		63 ± 4	83 ± 1
$\Delta S^{\ddagger}$, J/K mol		9 ± 15	55 ± 5
$\Delta V^{\ddagger}$, cm^3/mol		9.1 ± 0.9	6.7 ± 0.3

[a] Experimental rate data are reported in Figures 4 and 5. For experimental conditions see these figures.

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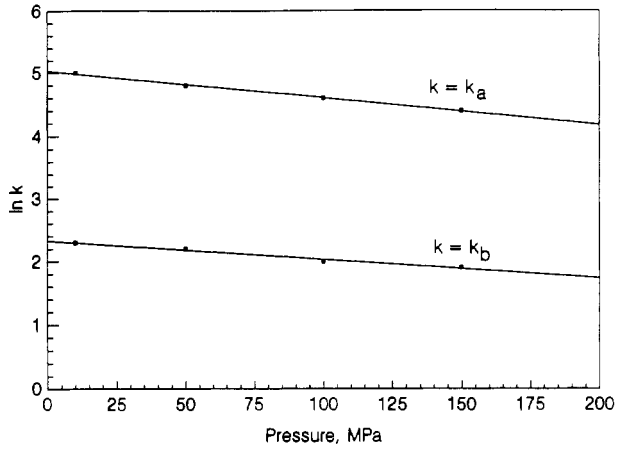

Figure 6. Plots of ln k versus pressure for the substitution of B_{12}–H_2O^+ by thiourea. For experimental conditions, see Figure 5.

Table II. Kinetic Data for the Reaction of DMTU with B_{12}–H_2O^+ at Various Temperatures[a] and Pressures[b]

T, °C	p, MPa	[DMTU], M	k_{obs},[c] s^{-1}	k_a, M^{-1} s^{-1}	k_b, s^{-1}
15.0	0.1	0.052	17.5 ± 0.3	40.7 ± 0.7	15.4 ± 0.1
		0.10	19.5 ± 0.2		
		0.15	21.6 ± 0.3		
		0.20	23.5 ± 0.4		
20.0	0.1	0.052	28.0 ± 0.4	56 ± 3	25.3 ± 0.5
		0.10	31.1 ± 0.6		
		0.15	34.1 ± 0.4		
		0.20	36.3 ± 0.5		
	10	0.050	25.4 ± 0.9	106 ± 8	20.5 ± 0.8
		0.075	28.8 ± 0.9		
		0.10	31.3 ± 0.8		
		0.125	33.4 ± 1.0		
	50	0.050	22.2 ± 0.3	96 ± 6	17.3 ± 0.5
		0.075	24.7 ± 0.7		
		0.10	26.6 ± 0.5		
		0.125	29.6 ± 1.0		
	100	0.050	17.8 ± 0.7	89 ± 7	13.5 ± 0.6
		0.075	20.5 ± 0.8		
		0.10	22.0 ± 0.8		
		0.125	24.7 ± 0.9		
	150	0.050	15.1 ± 0.6	71 ± 5	11.7 ± 0.4
		0.075	17.3 ± 0.4		
		0.10	18.6 ± 0.2		
		0.125	20.6 ± 0.4		
25.0	0.1	0.052	44 ± 1	115 ± 2	37.9 ± 0.2
		0.10	49.3 ± 0.8		
		0.15	55.0 ± 0.8		
		0.20	61 ± 1		
30.0	0.1	0.052	73 ± 1	217 ± 15	61 ± 2
		0.10	81 ± 2		
		0.15	95 ± 1		
		0.20	104 ± 3		
35.0	0.1	0.052	113 ± 3	312 ± 15	98 ± 2
		0.10	130 ± 2		
		0.15	146 ± 1		
		0.20	159 ± 2		
$\Delta H^{\ddagger}$, kJ/mol				78 ± 6	65 ± 1
$\Delta S^{\ddagger}$, J/K mol				55 ± 20	4 ± 5
$\Delta V^{\ddagger}$, cm^3/mol				6.7 ± 1.0	10.0 ± 0.8

[a] Experimental conditions: $[B_{12}] = 0.70 \times 10^{-4}$ M, pH = 4.0, λ = 570 nm, ionic strength = 0.10 M. [b] Experimental conditions: $[B_{12}] = 1.40 \times 10^{-4}$ M, pH = 4.0, λ = 370 nm, ionic strength = 0.10 M. [c] Mean value of at least 10 kinetic runs.

group of the nucleotide side chain is far away from the reaction center.[7,8] The latter distribution may cause some specific site interaction with the entering nucleophile in order to account for the observed kinetic saturation effect. There seems to be no correlation whatsoever between the values of k_a, on the one hand, and the overall formation constant K_1, the charge on the entering ligand, and the nucleophilicity of the entering ligand, on the other hand. For instance, k_a has a maximum value of ca. 1400 M^{-1} s^{-1} at 25 °C for the reaction with SCN^- (n_{Pt} = 6.65) and a minimum value of 3 M^{-1} s^{-1} at 25 °C for the reaction with SO_3^{2-} (n_{Pt} = 5.79), whereas the values for log K_1 are 3.1 and 7.3, respectively.[13] Furthermore, the value of k_a increases from 34 to 184 M^{-1} s^{-1} at 25 °C in going from PY (n_{Pt} = 3.31) to TU (n_{Pt} = 7.17), whereas log K_1 remains constant at 1.3 (ref 7 and this work). Similarly, there is no correlation of k_a with the values of $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ for a wide range of nucleophiles.[6,7,12,13] However, there seems to be some correlation between the pK_a values of a series of primary amines and the corresponding $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ values for their reactions with B_{12}–H_2O^+.[12] This was interpreted as evidence for specific hydrogen bonding between a functional group not more than two carbon atoms away from the coordinating amino functionality and the amide side chains of the corrin ring.[13] These trends are all in line with an I_d mechanism in which the most important kinetic contribution comes from the breakage of the B_{12}–H_2O bond, such that the properties of the entering nucleophiles play a minor role.

In the case of a few nucleophiles, viz. PY, TU, DMTU, SCN^-, SO_3^{2-}, $S_2O_3^{2-}$, and I^-, kinetic evidence for a reverse aquation reaction (k_b) was observed.[12,20-22] There is a good correlation between the value of k_b and the associated $\Delta H^{\ddagger}$ values, viz. a decrease in k_b is accompanied by a significant increase in $\Delta H^{\ddagger}$. This is in excellent agreement with the concepts of an I_d mechanism since B_{12}–L bond breakage will control the rate of the reverse aquation reaction and must strongly depend on the nature and binding mode of L.

Volumes of activation for the forward (k_a) and reverse (k_b) substitution reactions in (1) are summarized in Table III for a series of neutral entering ligands. The value for $\Delta V^{\ddagger}$ (k_a), L = TU, found in this study is significantly larger than that reported elsewhere.[19] In our work we observed that the effect of pressure on k_{obs} as a function of [L] is not that large and requires a very systematic series of measurements. In addition, it was easier to separate the pressure dependencies of k_a and k_b from such data when working in a wider pressure range. Our modified high-pressure stopped-flow system allowed us to work up to 150 MPa compared to 100 MPa in other studies,[7,19] which improved the quality of the data significantly. Our value of 9.1 ± 0.9 cm^3 mol^{-1} seems to be in good agreement with those found for PY and DMTU. These results are very typical for an I_d type of mechanism where we mainly observe the lengthening of the B_{12}–H_2O bond in the transition state, which should be independent of L.[1-4] Furthermore, since $k_a = k_2K_1$, $\Delta V^{\ddagger}(k_a) = \Delta V^{\ddagger}(k_2) + \Delta \bar{V}(K_1)$ simplifies to $\Delta V^{\ddagger}(k_a) \approx \Delta V^{\ddagger}(k_2)$ since $\Delta \bar{V}(K_1) \approx 0$ for neutral L. Thus the observed $\Delta V^{\ddagger}(k_a)$ values should all be very similar and independent of L. In addition, they should be significantly smaller than those reported for a limiting D mechanism. This is in fact the case, since $\Delta V^{\ddagger}$ values for ligand substitution on $Co(TMPP)(H_2O)_2^{5+}$ and $Co(TPPS)(H_2O)_2^{3-}$, where TMPP = *meso*-tetrakis(4-*N*-methylpyridyl)porphine and TPPS = *meso*-tetrakis(*p*-sulfonatophenyl)porphine, are 14 ± 4 and 15.4 ± 0.6 cm^3 mol^{-1}, respectively,[23,24] and have been assigned to a limiting D mechanism. Finally, the effect of pressure on the limiting rate constant observed for the reaction with PY must according to the mechanism in (5) represent $\Delta V^{\ddagger}(k_2)$, which has the value +7.1 ± 1.0 cm^3 mol^{-1}. This is once again in excellent agreement with that expected for a dissociative interchange of ligands in the precursor species.[1-4] In fact, in our earlier interpretation of these data,[6] the latter value was incorrectly assigned to the dissociation of the water molecule in B_{12}–H_2O^+ according to a limiting D mechanism. The value is definitely too low for such a mechanism,[23,24] but we were misled by the unexpected nonlinear concentration dependence of k_{obs}.[7]

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Table III. Summary of Available Kinetic Data for the Substitution of B_{12}–H_2O^+ by Several Ligands for the Overall Reaction B_{12}–$H_2O^+ + L \rightleftharpoons B_{12}$–$L^+ + H_2O$ (k_a, k_b)

L	k_a, $M^{-1}\,s^{-1}$	$\Delta H_a^{\ddagger}$, kJ/mol	$\Delta S_a^{\ddagger}$, J/K mol	$\Delta V^{\ddagger}(k_a)$, $cm^3\,mol^{-1}$	k_b, s^{-1}	$\Delta H_b^{\ddagger}$, kJ/mol	$\Delta S_b^{\ddagger}$, J/K mol	$\Delta V^{\ddagger}(k_b)$, cm^3/mol	$\Delta \bar{V}$,[a] cm^3/mol	ref
PY	34.2 ± 0.9	73 ± 5	29 ± 17	8.7 ± 1.4	2.28 ± 0.06	86 ± 3	50 ± 10	16.9 ± 0.8	−8.2 ± 2.2	7
TU	223	69 ± 2	32 ± 6	3.6 ± 0.5	15.2	85 ± 3	61 ± 11	6.3 ± 2.5	−2.7 ± 3.0	19
	184 ± 5	63 ± 4	9 ± 15	9.1 ± 0.9	15.5 ± 0.4	83 ± 1	55 ± 5	6.7 ± 0.3	2.4 ± 1.2	*b*
DMTU	115 ± 2	78 ± 6	55 ± 20	6.7 ± 1.0	37.9 ± 0.2	65 ± 1	4 ± 5	10.0 ± 0.8	−3.3 ± 1.8	*b*
SCN^-	2.3×10^3	72 ± 2	59 ± 4		1.8	72 ± 2	2 ± 8			21
I^-	1.4×10^3	67 ± 0.4	20 ± 1	5.5 ± 0.8	35 ± 4			11.5 ± 1.6	−5.8 ± 2.3	21, 22
$S_2O_3^{2-}$	200 ± 20	63 ± 3	8 ± 8	6.0 ± 0.8	3.5×10^{-2}	80 ± 2	−3 ± 8	−2.7 ± 0.5[c]		19, 21
SO_3^{2-}	3.3 ± 0.1	79.9 ± 0.5	33 ± 1		4×10^{-3}	96 ± 7	32 ± 22			12

[a] $\Delta \bar{V} = \Delta V^{\ddagger}(k_a) - \Delta V^{\ddagger}(k_b)$. [b] This work. [c] In a 30% acetonitrile–water mixture.

The values of $\Delta V^{\ddagger}$ for the reverse aquation reaction for L = TU and DMTU in Table III are also very typical for an I_d mechanism. The value for L = PY is misleadingly high and may be related to specific interaction of the B_{12} molecule with pyridine, which causes the overall reaction volume to be significantly more negative than for the other neutral ligands (see Table III) and will result in a large volume increase during the dissociation of PY. In fact, the overall $\Delta \bar{V}$ calculated from the kinetic data is close to zero for L = TU and DMTU and was confirmed by recording spectra of equilibrium mixtures as a function of pressure as done before.[6]

We conclude that the new data reported in this study clearly confirm the operation of an I_d mechanism for ligand substitution reactions of B_{12}–H_2O^+ and B_{12}–L^+. The misleading observations in the substitution reaction with PY must be related to a very specific interaction of B_{12} with PY. This interaction may involve the π character of the pyridine ligand and must account for the relatively strong precursor formation step and for the very large $\Delta V^{\ddagger}$ value found for the reverse aquation reaction. Nonlinear dependencies of k_{obs} on the entering ligand concentration were recently also found for a series of neutral nucleophiles, viz. hydroxylamine, methyl glycinate, 4-methylpyridine, imidazole, and histamine,[20] with precursor formation constants ranging from 0.2 to 3.1 M^{-1} at 25 °C. The authors found no specific correlation to account for these effects. This aspect requires further investigation and will be studied in more detail in our laboratory.

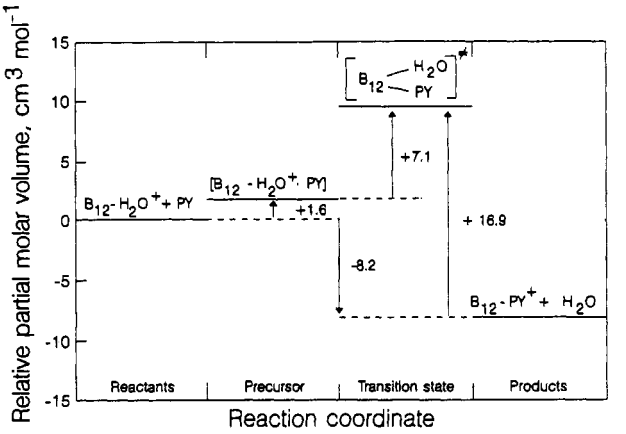

Figure 7. Corrected volume profile for the reaction B_{12}–$H_2O^+ + PY \rightleftharpoons B_{12}$–$PY^+ + H_2O$ according to the scheme presented in (5).

Whatever the explanation for this peculiar behavior will be, ligand substitution on B_{12} follows an I_d mechanism, and the corrected volume profile for the reaction with PY is therefore reported in Figure 7.

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